SOME STUDIES ON THE PRODUCTION AND FUNCTION OF THE THORACIC DUCT LYMPHOCYTES

with observations on the effect of colchicine on these and other leucocytes

A Thesis submitted for the Degree of
MASTER OF SCIENCE
of
The University of Edinburgh

by

Rose Krishna Madan, M.B.B.S.,(Punjab)

July, 1958

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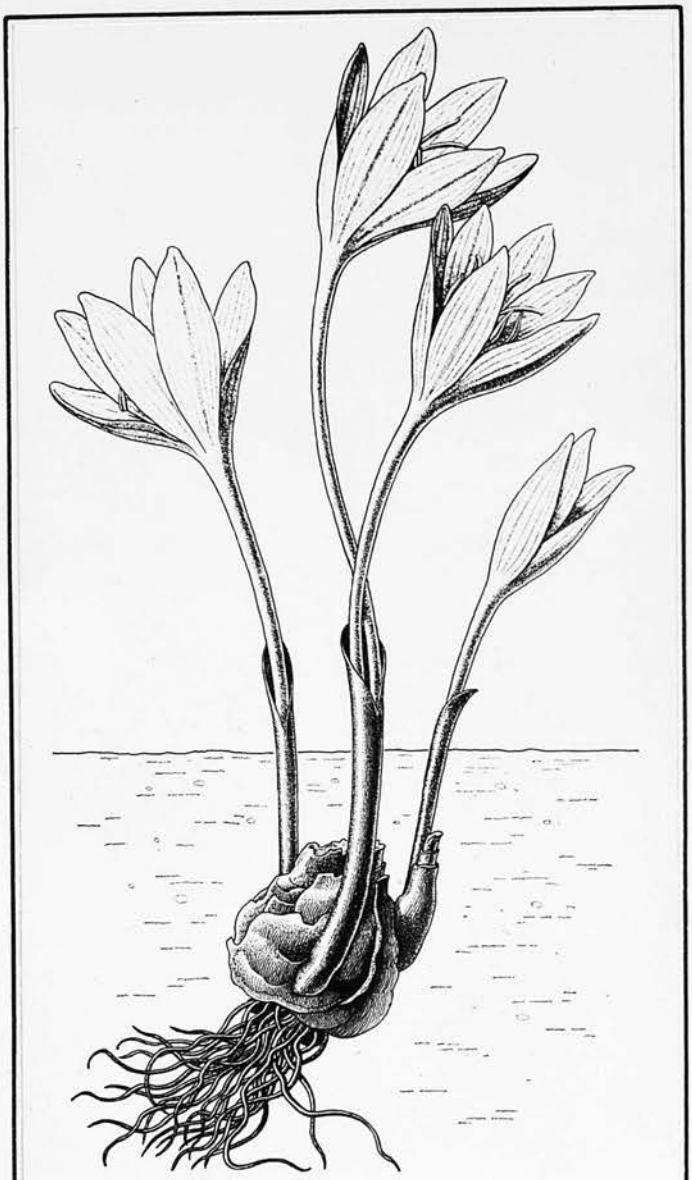

MEADOW SAFFRON, a pink or purplish wildflower which blooms in the fall, is the source of colchicine, a substance used to relieve the pain of gout. Colchicine is obtained from the seeds and bulbous root of the plant. The efficacy of the extract was discovered by Alexander of Tralles in 550 A.D. It is still the most effective treatment for the pain of gout.

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INTRODUCTION AND PLAN OF EXPERIMENTS

Part I: The effect of colchicine on thoracic duct lymph

The number of lymphocytes entering the blood by way of the thoracic and other lymph ducts is sufficient to replace the total number of lymphocytes many times a day (Yoffey, 1936; Sanders, Florey and Barnes, 1940; Hughes, May and Widdicombe, 1956; Whaler and Widdicombe, 1956; Gowans, 1957). It is uncertain whether the majority of these lymphocytes found in the thoracic and right lymph ducts are newly formed cells or not. A study of the relevant literature shows that some workers (e.g. Yoffey and Drinker, 1939a) consider that a circulation of lymphocytes between blood and lymph occurs on a very limited scale and that the cells of the lymph ducts are mostly newly formed. Others (e.g. Farr, 1951; Gowans, 1957) have reported work that is consistent with a considerable recirculation of lymphocytes from blood to lymph; in this case, newly formed cells in the lymph ducts would be few. It was thought that the alkaloid, colchicine, might be of some help in providing an answer to this question because of its well-known action of arresting dividing cells at the metaphase stage. If the majority of the lymphocytes were newly formed, this colchicine-inhibition of cell division might reduce appreciably the number of cells coming down the duct or cause the appearance of a large number of cells in arrested mitosis.

Part II: The effect of colchicine on peripheral blood leucocytes

During the course of this work on the lymphocyte and colchicine changes in the level of granulocytes in peripheral blood were revealed which demanded more attention on their own merit.

Dixon (1908 and 1912) working on rabbits, Fagraeus and Gormsen (1953) on rats and Harm (1953) on rabbits had reported that following the administration of colchicine in unanaesthetised animals there was an initial leucopaenia affecting both the granulocytes and the lymphocytes for a period of an hour or so, followed by a leucocytosis which reaches its peak within six to ten hours, and for this the granulocytes were held mainly responsible with little or no effect on the lymphocytes. In the present work this reaction was confirmed but a striking deviation from this typical pattern was observed in operated and anaesthetised rabbits. It was seen that the initial granulocytopaenia after colchicine tended to persist with little or no recovery of the granulocytes; even towards the end of a period of ten hours there was no evidence of any granulocytosis. It appeared as if colchicine was acting in combination with the operative stress and the anaesthesia or with the anaesthesia only, to abolish or delay the onset of the leucocytosis.

Part III: The relation of lymphocytes and antibody formation

The lymphatic tissue of the body has been implicated in antibody formation (e.g. Roberts and Gowans, 1958). It would seem that, under some circumstances, the thoracic duct lymphocyte of rabbits could itself be an antibody producer (Wesslen, 1952; Holoub, 1957). Further work appeared to be needed to substantiate and clarify this relation between antibody formation and the thoracic duct lymphocyte. Accordingly two studies were made. In one, the technique using colchicine, developed in the work planned as in Part I above, was extended to antibody-producing animals. In the other, thoracic duct lymphocytes from antibody-producing rabbits were transplanted to recipient unimmunized rabbits. It was hoped that in this way, antibody formation could be associated in these experiments with a single cell type and a function be ascribed to the lymphocyte.

Part I: The effect of colchicine on thoracic duct lymph

EXPERIMENTAL METHODS

1. ANIMALS USED

Stock rabbits of various strains and of either sex were used, weighing from 2.0 to 3.0kg.

2. OPERATIVE TECHNIQUES

a) Anaesthesia. The animals were anaesthetised with Phenobarbitone Sodium B.P. (Abbott "Veterinary Nembutal" containing 60mg. per ml. of Phenobarbitone Sodium B.P.) It was diluted with about 3.0ml. of saline just before use for the initial injection and subsequently the amount of saline used for dilution was equal to the amount of lymph collected in the preceding hour.

The initial dose of Nembutal intravenously was usually 45.0mg. per kg. body weight injected into the right ear vein and the maintenance dose of about half this was repeated every hour or every one and half hour. This kept the animals in a state of light anaesthesia throughout the period of the experiment. Occasionally a wisp of ether had to be given just before making the skin incision for exposing the thoracic duct, but ether was otherwise never used. It has been shown by Rous (1908a) that struggle and increased muscular activity produces an increase in the lymphocyte output through the thoracic duct. As ether tends to produce an

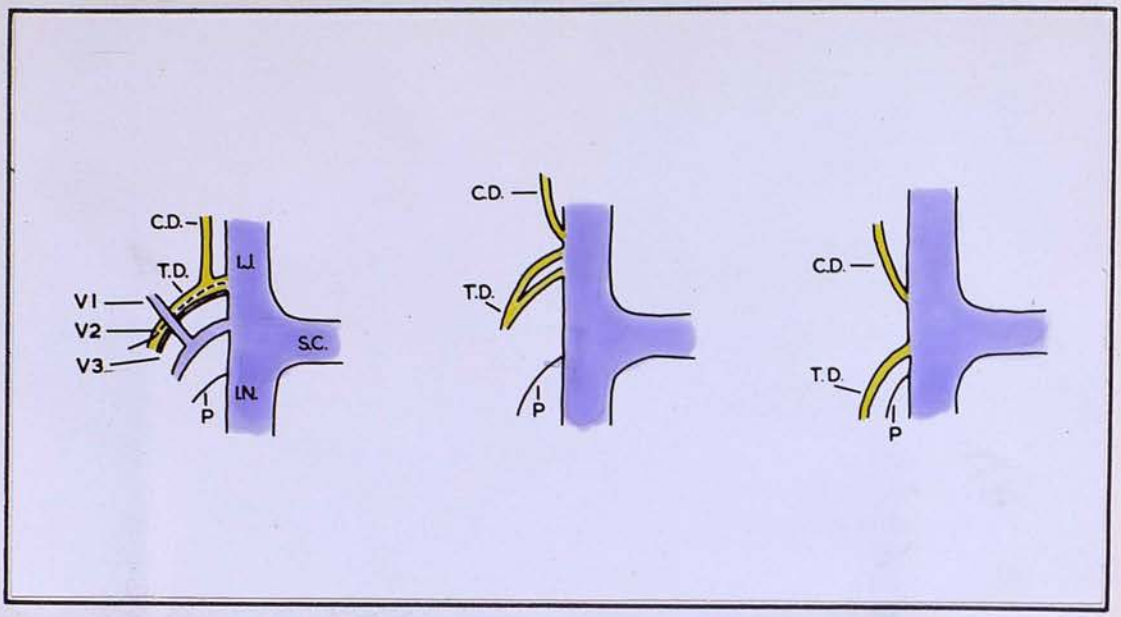

Fig. 1. Diagram showing the ways in which the thoracic duct may join the internal jugular vein in the rabbit.

Left: The left cervical duct joins the thoracic duct which enters just above the junction of internal jugular and subclavian veins.

Middle: The thoracic duct divides and each branch enters the internal jugular vein separately.

Right: The thoracic duct enters the internal jugular vein in close proximity to the pleura.

I.J. = Internal jugular vein.

S.C. = Subclavian vein.

I.N. = Innominate vein.

T.D. = Thoracic duct.

C.D. = Left cervical duct

P. = Pleura

V1 = Small vein crossing the thoracic duct.

V2 = Small vein crossed by the thoracic duct (indicated by a dashed line).

V3 = Small vein accompanying the thoracic duct.

V1, V2 and V3, although only indicated in one drawing, are constantly present.

initial struggle, this would certainly vitiate the rate of lymph flow and its cellular content.

b) Exposure and anatomy of the junction between the thoracic duct and the internal jugular vein. The thoracic duct was exposed in the neck as described by Kindwall (1927). The fur was clipped from the front of the neck and the skin was dabbed with 70% alcohol. A skin incision of about 3.0cm. in length was made in the midline from the angle of the rib upwards. The three deep layers of muscles connecting the clavicle to the sternum and the humerus were then cut just to the left of the midline, taking every precaution to prevent unnecessary bleeding. The junction of the subclavian and the jugular veins was seen to be covered with a thick white fascia which was removed very carefully fibre by fibre with the aid of dissecting spectacles, this being necessary owing to the close proximity of the pleura. The anatomy of the thoracic duct in this region conformed to one or other of the patterns shown in Fig. 1.

In four experiments the duct was cannulated, according to Sanders, Florey and Barnes (1940), indirectly by way of the jugular vein, after ligature of all the afferent and efferent vessels near the orifice of the duct whereby a sac is formed by the jugular vein into which only the thoracic duct empties. A polythene cannula filled with heparin was introduced into the sac and lymph collected into centrifuge tubes containing 3 drops of a solution of

heparin and the tubes kept at a temperature of 4°C. to minimize cell lysis. This method of cannulation of the duct was found to be difficult and uncertain for, at the best of times, some tiny blood vessel would be left unligatured, giving rise to blood-stained lymph. A direct cannulation of the duct with a polythene cannula of the appropriate size was therefore attempted next. Although the samples of lymph thus obtained were very satisfactory there was a success rate of less than one in five in the hands of the present experimenter. This technique of direct cannulation of the thoracic duct was therefore abandoned in favour of the following method.

With experience, it was found to be quite easy to ligature all the small blood vessels in the immediate vicinity of the thoracic duct and to cut the duct to allow a flow of lymph. By continuously pipetting lymph from the pool so produced the total output of the thoracic duct could be obtained free of blood. The use of the cautery to obtain an operation area free from bleeding, as suggested by Wesslén (1952), was not thought necessary because simply ligaturing the blood vessels was as good as cauterization and possibly far less traumatizing. It was rare that lymph samples contaminated with blood were obtained; such samples were always discarded. There are two objections to this 'pool' technique for collecting lymph; first; a possibility of contamination of lymph with

exudative cells (granulocytes and monocytes) from the operated area and secondly a possibility that the lymph was diluted with tissue fluid. Microscopic examination of the lymph smear only on rare occasions revealed even so much as a single granulocyte in the whole smear. The problem of dilution of lymph with tissue fluid is negligible considering the small area of the operation field, this being never more than 1.5 x 2.0cm. approximately, and the large amounts of lymph collected, ranging from about 2.0 to 10.0ml. per hour.

Clotting of the lymph during the collection was found to be no problem. Thoracic duct lymph will certainly clot if it is allowed to remain in the pool for some time, (the clotting time of a sample of thoracic duct lymph was found to be 4 minutes). But a spontaneous flow of, from about 2 to 10ml. of lymph per hour, which was pipetted off as soon as it filled the pool, kept the duct unobstructed for the entire period of the experiment. In no experiment could any indication be obtained that the duct was obstructed by clots.

Thus it appears to be quite reasonable to assume that the results of experiments on the lymph obtained by this 'pool' technique can safely approach in value the results of experiments obtained by direct cannulation of the duct.

Throughout the operation and the subsequent periods of lymph collection the animal was kept warm. The tissues

surrounding the pool of lymph were covered by cotton wool moistened with normal saline.

3. COLCHICINE

Colchicine* was dissolved in water in proportions of 5mg. per ml. of water and was then injected into the right ear vein, leaving the left ear vein to obtain samples of blood.

The dose of colchicine required was calculated on the basis of the dose used by Dixon and Malden (1908) who studied its effects on the blood leucocytes in rabbits. The dose used by them as a single injection was about 5.0mg. per kg. body weight injected subcutaneously. Since it was desired to obtain the effect of colchicine as early as possible, the injection was given intravenously and half the above dose was used, i.e. 2.5mg. per kg. body weight. When 5.0mg. per kg. body weight of colchicine was injected it tended to produce diarrhoea in some of the animals. With 2.5mg. per kg. no observable ill effects were produced either immediately or in animals kept for weeks afterwards. Approximately the same order of dose was used in mice by Roberts, Florey and Joklik (1952) for obtaining the effective arrest of mitosis in metaphase in the liver cells. It will be shown that, in the rabbit, 2.5mg. per kg. body weight produced arrest of mitosis of some thoracic duct cells in metaphase.

*Colchicine, B.D.H.

4. COLLECTION AND EXAMINATION OF LYMPH

a) Volume. Lymph was collected in hourly or in two hourly volumes, from the pool prepared by dissecting and then dividing the thoracic duct in the neck. A Pasteur pipette was used to remove the lymph from the pool as it filled. Care was taken to avoid air bubbles which tend to break up the cells. The collecting container consisted of a graduated 10ml. centrifuge tube so that lymph volumes could be measured directly. The tube contained 2 to 3 drops of a solution of heparin containing 1,000 units/ml.* to prevent clotting of the lymph. The centrifuge tubes were placed in a vessel containing ice, so that the temperature of lymph could be kept low, this helps to preserve the cells. The hourly volumes of lymph were recorded.

The tubes were then gently agitated, and total and differential counts made.

b) Total cells per hour. Leucocyte counts were always made completely in duplicate on each specimen. The counts were always within 10% of each other.

The lymph was diluted 1 in 100 in a red cell counting pipette, using solution of 0.172% brilliant cresyl blue containing 0.04% sodium cyanide, according to the method of Hechter and Johnson (1949). Cresyl blue-cyanide stains the leucocytes blue and haemolyzes the erythrocytes. After rotating the pipette for a minute, counts were made

* 'Liquemin' Heparin "Roche" Solution

in a standard haemocytometer chamber by counting the number of cells in 0.4 cmm. (as for white blood cells) under the low power and then multiplying the results by 250 to obtain the number of cells per cubic millimetre. From the mean leucocyte values per cubic millimetre and the volume of lymph collected hourly, the number of cells delivered per hour by the thoracic duct was computed.

c) Differential leucocyte count. An accurate method for determining the relative numbers of large and small lymphocytes in the thoracic duct lymph was necessary and a number of methods were put to test before a satisfactory technique was discovered. Rous (1908c) first pointed out the difficulty of making satisfactory films of lymph due to the lack of 'body' in it as compared to blood. To overcome this difficulty small amounts of lymph were centrifuged at a slow speed (600r.p.m.) so as not to break up the cells. The lymph plasma was removed and a small amount of rabbit serum was added. Smears were then prepared, fixed in methyl alcohol and stained by May-Grunwald Giemsa Method (Dacie, 1956). An accurate count of these preparations was not possible because of the uneven distribution of the cells in the smears, the large cells tending to aggregate towards the tail end of the film; moreover great numbers of the cells in the smear were smudged and this tendency for the cells to break up was accentuated in lymph obtained from animals treated with colchicine. This method

had therefore to be abandoned.

It was thought that Woodruff's method (1952), which he used on blood for differential counts, might help. Fine lines of 0.5mm. thickness and 10.0mm. in length were made by means of a draughtman's pen on a glass slide from the lymph specimen, and stained as for lymph smears. An attempt was made to count all the cells in a line under the high power of the microscope. The results so obtained in one line could not be repeated on the other line of the same sample of lymph.

Next the supravital method of staining as modified by Schwind (1950) was tried, using Neutral Red and Pinocyanole. This method was not successful because the structure of the nuclei of the cells was not clearly revealed nor was it possible to differentiate the abnormal cell nuclei which appeared in the thoracic duct lymph of colchicine-treated animals.

It appeared that the most accurate way for a differential cell count would be to count cells in the counting chamber of the haemocytometer and to use a diluting solution which would help to stain the nuclei and reveal their details. The ordinary white cell diluting fluid was of little help. Hechter and Johnson (1949) in their experiments to show the in vitro effect of adrenal-cortical extract upon lymphocytolysis used a diluting fluid solution of 0.172% brilliant cresyl blue containing 0.04% sodium cyanide.

Comparative studies using first brilliant cresyl blue-

cyanide and then 1% acetic acid as diluent for a given sample of the lymph revealed similar total counts.

If the count was not to be made immediately, samples of lymph were drawn into the red cell pipettes, diluted with cold cresyl blue-cyanide and the pipette containing the diluted suspension placed in the refrigerator at 2°C until a cell count was actually made. This was done, at the latest, the following morning.

The differential leucocyte counts were made in a standard haemocytometer in duplicates i.e. 2 aliquots were taken into separate pipettes and a count made from each. From 100 to 300 cells were counted for each aliquot. Absolute numbers of each variety of cells coming down the duct were also calculated from the count using cresyl blue-cyanide, and the volume of the lymph per hour.

d) Distinction between large and small lymphocytes.

No direct measurements of cell diameters were carried out, but an arbitary and approximate distinction between large and small lymphocytes was made on the following bases:-

Any lymphocyte which was twice the size or more of the typical small lymphocyte was considered as a large lymphocyte. The small lymphocyte is, of course, by far the commonest cell present in thoracic duct lymph, is of almost uniform size and is the smallest cell present in the lymph. When lymph was stained with brilliant cresyl blue-cyanide, the large lymphocyte, as judged by this criterion, looked much

paler in colour and had much greater amounts of cytoplasm than the small lymphocyte. The small lymphocyte when stained in this way appeared as a dark round spot of ink with very small amount of cytoplasm. The size of the comparatively few cells in between a small and a large lymphocyte could be considered to be medium-sized lymphocytes, but were counted along with the small lymphocytes.

It should be noted that a given sample of lymph, when examined using these criteria, gave a repeatedly consistent percentage of large and small lymphocytes. As noted above, the counts of the relative proportion of large and small lymphocytes were done in duplicates on each sample and the result obtained differed by no more than from 0 to 5%.

e) Lymph smears. Lymph was centrifuged at 600r.p.m. for 5 minutes, the lymph plasma was removed and a small volume of rabbit's serum was added. The tube was then gently agitated until the pellet of cells broke up. Smears were made, and after drying, were fixed in methyl alcohol and stained by May-Grunwald Giemsa Method. These smears were examined under the oil-immersion objective in order to study the morphology of the lymphocytes especially those showing any mitotic abnormality. This was possible because, in the smear, the cells spread out and their structure could be seen clearly. As explained above, no estimates of the relative numbers of large and small

lymphocytes were possible in smears.

5. EXAMINATION OF THE PERIPHERAL BLOOD

a) Total leucocyte counts. Blood for examination was obtained from the marginal ear vein of the side not used for the injection of either colchicine or Nembutal. These were performed, always in duplicate, in the usual way in the haemocytometer chamber by counting the number of cells in 0.4 cubic millimetre, and then multiplying by 50 to obtain the number of cells per cubic millimetre of blood.

b) Differential leucocyte counts. These were performed, always in duplicate, and by counting 200 cells in each smear. Blood smears were prepared, dried, fixed in methyl alcohol and stained by May-Grunwald Giemsa method. The lymphocytes were differentiated into two groups, one comprising the small and the medium-sized cells, and the other, the large lymphocytes. The criteria used were as given above. It was found that, in blood smears, the lymphocytes did not break up and were distributed evenly along the length of the smear. A record of any abnormal types of cell or cell fragments seen in the film was also made. The absolute numbers of the various cells per cubic millimetre of blood were determined from the total and differential counts.

c) Smears of lymphocytes separated from blood. The lymphocytes were separated from the blood by the method devised by Jago (1956). Smears were prepared, stained by

May-Grunwald Giemsa method. An examination of these lymphocytes was made particularly with a view to finding abnormal lymphocytes.

6. EXAMINATION OF THE SPLEEN

a) Spleen weight. The spleen was removed immediately the animal was sacrificed. It was freed of the fat and the surrounding connective tissue and then weighed on a torsion balance to the nearest milligram.

b) Splenic imprints. These were prepared and stained by May-Grunwald method. Examination of the imprints for any abnormal figures appearing in the lymphocytes was made.

c) Histological sections. Pieces of the spleen were fixed in 10% buffered formalin solution, washed, dehydrated, cleared and embedded in paraffin wax. Sections at about 6μ were cut. (Carleton, 1957).

7. PROTOCOL OF A TYPICAL EXPERIMENT

Black and white rabbit. Female. Weight 2.05kg. Date 1.2.58.

Time	
11.10 a.m.	Sample of blood for total and differential leucocyte counts taken from the left ear vein.
11.15 a.m.	Nembutal 1.4ml. in 3.0ml. saline intravenously, into the right ear vein.
11.22 a.m.	Operation started.
12.15 p.m.	Blood sample.
12.18 p.m.	Nembutal 0.6ml. in 2.0ml. saline.

12.20 p.m.	Thoracic duct exposed and cut. Spontaneous flow of lymph which was collected by a Pasteur pipette into 15ml. centrifuge tube immersed in iced water.
12.35 to 1.35 p.m.	7.1ml. lymph collected. Total and relative numbers of small and large cells counted. Smears made.
1.35 p.m.	Blood sample.
1.36 p.m.	4.1mg. of colchicine (2.5mg./kg. body weight) in 0.8ml. of distilled water intravenously.
1.40 p.m.	Nembutal 0.8ml. in 7.0ml. saline.

Thereafter up to 11.35 p.m. hourly samples of lymph and of blood were collected and 0.6ml. of Nembutal diluted with the appropriate amount of saline was injected intravenously every one or one and a half hours.

11.40 p.m.	Animal sacrificed and spleen removed, weighed, imprints made and portions taken into formol saline for histology.

Part I: The effect of colchicine on thoracic duct lymph

RESULTS

The results of analysis of the samples of thoracic duct lymph and of peripheral blood obtained over a period of from 7 to 11 hours in 10 anaesthetized rabbits are given below and are shown in the accompanying Figs. 2. to 20. and Tables I. to VI. Colchicine was injected intravenously into 6 rabbits as a single dose of 2.5mg./kg. body weight, while the other 4 animals acted as controls and had no injection of colchicine. In each of the animals which were going to receive colchicine, the lymph was collected and analysed for a period of 1 hour. After this first hour, colchicine was immediately injected intravenously. Out of the 6 rabbits which received colchicine, one of the animals, No. T.D.1, died after 7 hours of lymph drainage and, throughout the period of the experiment, this animal remained very deeply anaesthetised. It may be noted , therefore, that the results of this experiment are not strictly comparable with the results of the remainder.

1. EFFECT OF COLCHICINE ON THE RATE OF LYMPH FLOW AND THE TOTAL NUMBER OF CELLS COMING DOWN THE THORACIC DUCT PER HOUR

a) Rate of lymph flow. It will be seen from Fig. 2. that, in the control, the flow of lymph varied little for

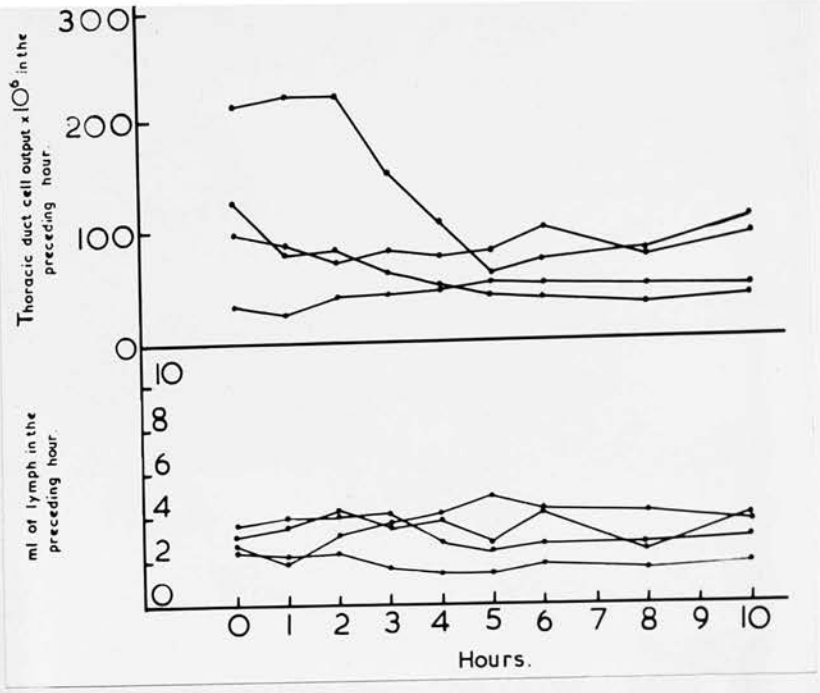

Fig. 2. The rate of thoracic duct lymph flow and the cell output per hour in 4 control rabbits.

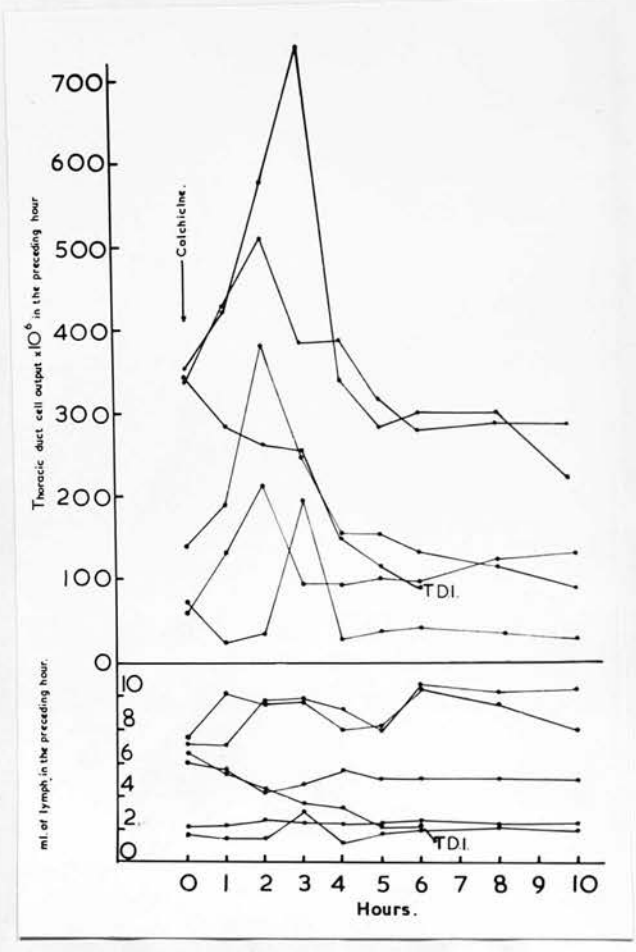

Fig. 3. The effect of colchicine on the rate of thoracic duct lymph flow and the cell output per hour in 6 rabbits.

the 11 hours of the experiment. Fig. 3. shows the flow in the rabbits receiving 2.5mg./kg. colchicine intravenously. In 4 out of the 6 animals, the initial flow before colchicine happened to be higher than those of the controls. The drug appeared to have no constant effect on the flow of lymph. In only 1 rabbit was there a consistent change: in this animal, No. T.D.1, the flow decreased for 7 hours at which time the animal died.

b) Cells per hour. It will be seen from Fig. 2. that the cell output per hour, during a period of 11 hours of lymph drainage, in the 4 control animals remains very nearly constant throughout the experimental period. In the rabbits receiving colchicine (Fig. 3.) the total number of cells per hour coming down the thoracic duct increases appreciably after the injection of the alkaloid and reaches a peak between 2 to 3 hours of colchicine administration, and thereafter declines almost immediately to reach a level usually slightly below the basal figure. This increase in the output of total cells is independent of the rate of flow of lymph, for it will be seen that in three of the experiments, in spite of no increase in the volume of the lymph during the hour, there is still a considerable increase in the output of cells during that hour. Furthermore, in the two experiments in which the hourly volume of lymph had increased, the relative increase in the output of the cells per hour far exceeds the level

Table I.

Table 1. shows the total number of small and large lymphocytes coming down the thoracic duct per hour and the percentage of large lymphocytes in normal animals.

Rabbit No.	Variables	Hours								
		0	1	2	3	4	5	6	8	10
C1	Small lymphocytes/hr. x 10^6	187.2	186.1	191.5	139.7	87.5	55.2	68.6	72.1	87.6
	Large lymphocytes/hr. x 10^6	8.6	17.0	11.0	12.1	8.4	4.2	4.9	7.2	10.3
	% Large lymphocytes	4.9	7.8	4.6	7.6	8.4	6.6	6.6	9.0	10.3
C2	Small lymphocytes/hr. x 10^6	100.4	80.9	58.1	73.8	70.8	69.8	89.5	55.7	67.0
	Large lymphocytes/hr. x 10^6	9.6	8.1	12.9	9.0	7.1	11.2	12.9	11.3	8.0
	% Large lymphocytes	8.0	8.1	17.1	10.9	9.1	12.5	12.5	17.3	10.7
C3	Small lymphocytes/hr. x 10^6	28.5	20.2	29.8	34.3	33.3	42.5	40.8	34.6	33.3
	Large lymphocytes/hr. x 10^6	5.0	5.3	11.5	8.5	9.7	12.5	10.8	13.7	14.2
	% Large lymphocytes	15.4	19.4	27.0	18.9	22.5	21.6	20.8	29.0	30.0
C4	Small lymphocytes/hr. x 10^6	137.5	76.5	75.3	59.5	41.6	35.2	31.7	26.0	28.8
	Large lymphocytes/hr. x 10^6	10.6	5.2	8.1	3.8	4.5	3.2	4.2	6.0	7.2
	% Large lymphocytes	7.2	6.3	9.7	6.0	9.8	8.0	11.0	18.5	20.6

which one would expect from the increase in rate of lymph flow. The curve showing the results in animal No. T.D.1 may be set aside since in this animal alone the flow consistently decreased for 7 hours, after which time the rabbit died. In one rabbit there was an initial slight drop in the number of cells per hour for the first 2 hours after colchicine but this animal showed an increase in the 3rd hour corresponding to the responses of the other animals. Comparing Figs. 2. and 3., it is obvious that colchicine increases by 50% or more the output of the cells from the thoracic duct sometime during the first 2 to 3 hours after the intravenous administration of colchicine, and that this increase is quite independent of the rate of lymph flow.

2. EFFECT OF COLCHICINE ON THE TYPES OF CELLS OF THE THORACIC DUCT LYMPH

a) Relative number of small and large lymphocytes.

Table I. gives the relative and absolute numbers of small and large lymphocytes passing down the thoracic duct in the 4 control experiments in which the rabbits received no colchicine. Fig. 4. presents these results graphically. Table II. and Fig. 5. give the corresponding results in the animals receiving colchicine. It can be seen that the absolute numbers of small and large lymphocytes vary little during the course of the experiment (10 hours) in the control animals compared with the noticeable increase

Table II.

Table 2. shows the total number of small and large lymphocytes coming down the thoracic duct per hour and the percentage of large lymphocytes in colchicine-treated animals.

Rabbit No.	Variables	Hours								
		Colchicine I.V.								
		0	1	2	3	4	5	6	8	10
TD1	Small lymphocytes/hr. x 10^6	327.9	259.6	250.8	247.0	144.8	107.2	84.5	-	-
	Large lymphocytes/hr. x 10^6	13.7	22.6	13.2	5.0	3.7	4.5	3.5	-	-
	% Large lymphocytes	4.0	8.0	5.0	2.0	2.5	4.0	4.0	-	-
TD2	Small lymphocytes/hr. x 10^6	127.5	179.5	355.2	232.9	138.6	147.6	119.2	111.6	78.8
	Large lymphocytes/hr. x 10^6	5.6	10.7	25.5	14.5	14.4	8.2	13.1	4.8	7.2
	% Large lymphocytes	4.0	6.0	7.0	6.0	9.0	5.0	10.0	4.0	8.0
TD3	Small lymphocytes/hr. x 10^6	53.1	135.5	200.5	85.8	84.8	93.1	95.6	118.1	126.4
	Large lymphocytes/hr. x 10^6	1.9	4.7	11.4	3.6	3.6	7.5	3.8	3.2	4.4
	% Large lymphocytes	4.0	3.3	5.3	4.5	4.1	7.1	3.5	2.6	3.4
TD4	Small lymphocytes/hr. x 10^6	69.3	23.9	31.6	186.0	27.0	35.3	39.6	35.6	28.8
	Large lymphocytes/hr. x 10^6	6.4	2.0	3.7	9.3	1.2	2.1	2.4	2.1	1.8
	% Large lymphocytes	8.4	8.0	10.6	4.0	4.3	5.7	5.7	5.4	6.0
TD5	Small lymphocytes/hr. x 10^6	324.9	379.9	535.8	706.3	337.2	248.0	262.5	265.6	186.4
	Large lymphocytes/hr. x 10^6	30.1	42.6	40.0	41.0	37.0	32.0	37.5	36.1	32.0
	% Large lymphocytes	8.5	9.4	6.1	5.9	6.5	11.0	12.0	11.5	14.0
TD6	Small lymphocytes/hr. x 10^6	310.5	375.2	466.7	347.9	355.6	300.0	255.0	270.3	286.8
	Large lymphocytes/hr. x 10^6	42.0	60.2	30.5	35.5	29.4	17.5	18.8	16.9	16.9
	% Large lymphocytes	11.4	13.5	6.6	9.2	6.8	4.9	6.6	5.5	5.8

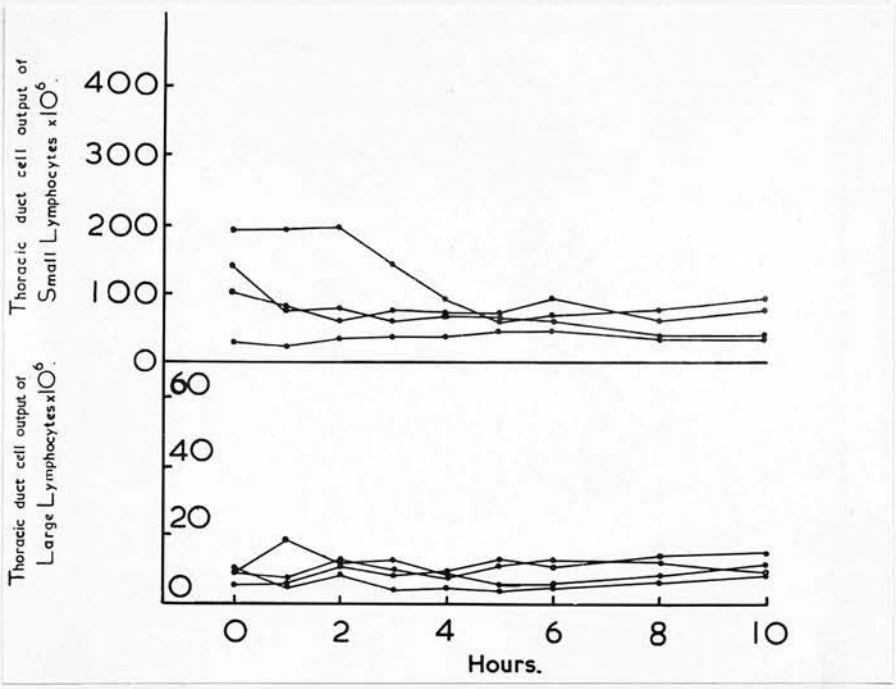

Fig. 4. The absolute numbers of small and large lymphocytes coming down the thoracic duct per hour in 4 control rabbits.

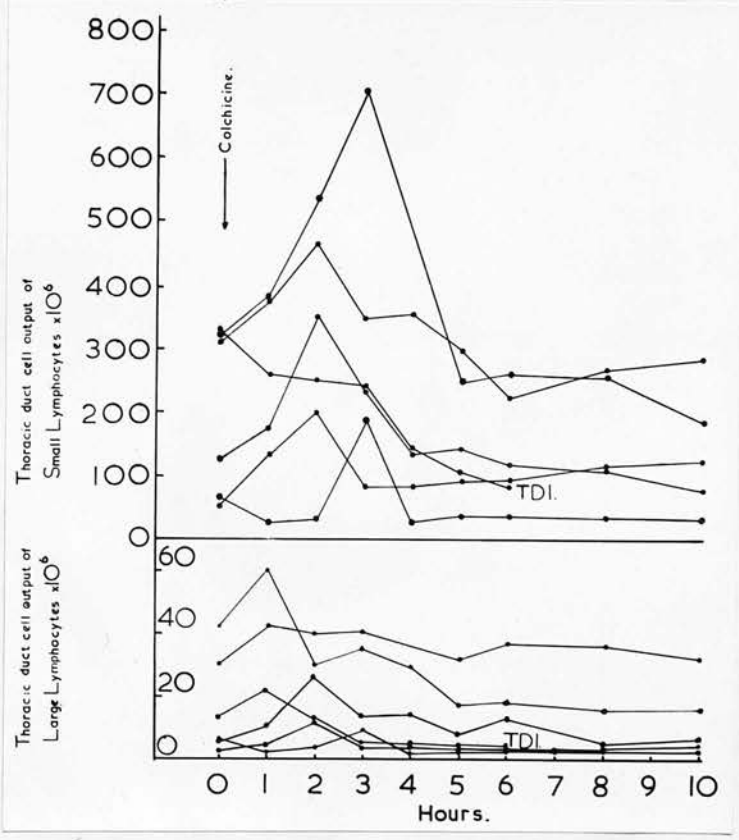

Fig. 5. The effect of colchicine on the absolute numbers of small and large lymphocytes coming down the thoracic duct per hour in 6 rabbits.

in both small and large lymphocytes 2-3 hours following colchicine. Colchicine may, therefore, be considered to affect the numbers of the small and large lymphocyte, causing an increase in both. It may also be noted that, in the control rabbits, the number of large lymphocytes relative to the number of small lymphocytes appears to increase after some hours drainage, reaching 20% and 30% in two animals after 10 hours.

b) Effect of colchicine on the morphology of the thoracic duct lymphocytes. The morphology of the cells of the thoracic duct lymph was studied in smears stained by the May-Grunwald Giemsa method.

The majority of the cells coming down the thoracic duct in 4 control rabbits were seen to be small sized cells about 9μ in diameter, having a dark round nucleus composed of dense masses of chromatin and surrounded by a pale rim of clear, basophilic cytoplasm with no vacuolation. These cells are considered as the small lymphocytes, and they corresponded with the description of the lymphocyte given, for example, by Yoffey and Courtice (1956). A few of the cells were less than twice the size of a small lymphocyte and may be called medium sized cells, having the same nuclear characters as the small lymphocyte but with slightly more cytoplasm around the nucleus. The cytoplasm of these cells was of a variable basophilia. As judged by counts made in the counting chamber, of lymph

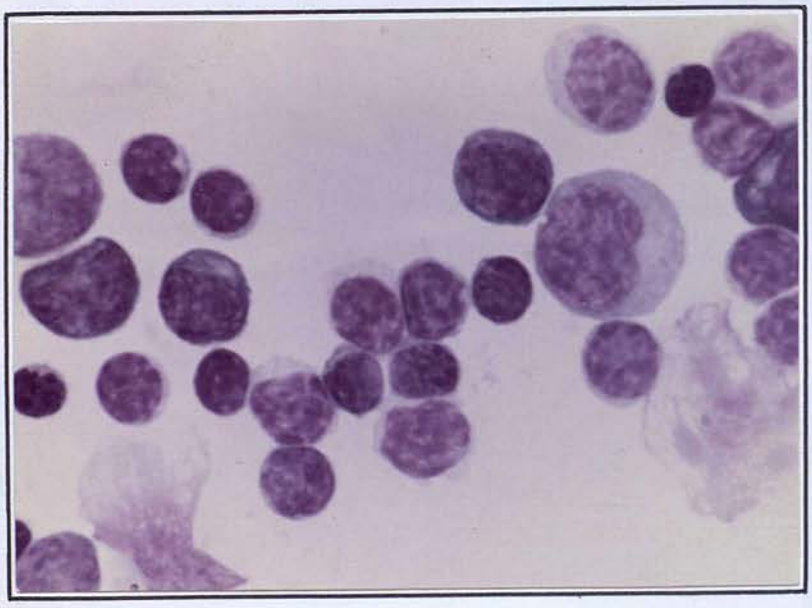

Fig. 6. Normal lymphocytes of the thoracic duct lymph. Lymph smear. May-Grunwald Giemsa x 1000.

stained by the brilliant cresyl blue-cyanide method, the small and medium sized lymphocytes form from 70 to 95% of the total thoracic duct lymphocytes. The remaining 5 to 30% of the thoracic duct cells is contributed by cells which are twice or more the size of a small lymphocyte, having a large nucleus and surrounded by large amounts of cytoplasm showing varying degrees of basophilia. Vacuolation of the cytoplasm was very rarely observed. These cells are called here large lymphocytes. Only on one occasion was a mitotic figure seen in the large lymphocyte out of the many thousands of thoracic duct lymphocytes seen.

Table II. and Fig. 6. show the relative number of small and large lymphocytes and the characters of the cells respectively, in the 4 control animals.

In animals that had received colchicine, the majority of the cells coming down the thoracic duct were found to be lymphocytes which exhibited no nuclear or other changes and had the characteristics of the thoracic duct cells of normal rabbits as described above (Figs. 8a to 8m). The other types of cells seen are the lymphocytes showing certain abnormalities of their nuclei and cytoplasm. Such cells will be considered as 'Abnormal' cells.

Normal cells

Table III. shows that apparently normal cells contribute from about 95 to 99% of the total cell population

Table III.

Table 3. shows the total number of cells (normal and abnormal cells coming down the thoracic duct per hour, and the percentage of abnormal cells* and of cells in mitosis, after the intravenous injection of colchicine.

Rabbit No.	Variables	Hours after Colchicine							
		1	2	3	4	5	6	8	10
TD1	Total cells/hr. x 10^6	282.2	264.0	252.0	148.5	111.7	88.0		
	Abnormal cells/hr. x 10^6	-	3.3	-	-	-	-	Animal	
	% Abnormal cells	-	1.3	-	-	-	-		
	% Cells in mitosis	-	0.86	-	-	-	-	died	
TD2	Total cells/hr. x 10^6	190.2	380.7	247.4	153.0	155.8	132.3	116.4	86.0
	Abnormal cells/hr. x 10^6	5.1	1.2	7.3	4.1	3.1	6.6	1.2	2.0
	% Abnormal cells	2.6	0.3	3.0	2.6	2.0	5.0	1.0	2.3
	% Cells in mitosis	1.3	0.3	1.5	1.7	0.6	1.2	1.0	1.5
TD3	Total cells/hr. x 10^6	140.2	211.9	89.4	88.4	100.6	99.4	121.3	130.8
	Abnormal cells/hr. x 10^6	1.7	1.9	0.6	0.6	0.6	0.6	0.9	-
	% Abnormal cells	1.2	0.9	0.7	0.7	0.6	0.6	0.8	-
	% Cells in mitosis	0.3	0.6	0.3	0.3	0.6	0.0	0.3	-
TD4	Total cells/hr. x 10^6	25.9	35.3	195.3	28.2	37.4	42.0	37.8	30.6
	Abnormal cells/hr. x 10^6	0.2	1.7	1.2	0.5	0.4	0.5	0.5	0.2
	% Abnormal cells	0.8	5.0	0.6	1.8	1.1	1.2	1.3	0.6
	% Cells in mitosis	0.0	2.5	0.2	0.6	1.1	0.6	0.7	-
TD5	Total cells/hr. x 10^6	422.5	575.8	747.3	337.2	280.0	300.0	301.7	218.4
	Abnormal cells/hr. x 10^6	3.5	9.8	9.8	9.2	12.3	8.0	7.4	7.0
	% Abnormal cells	0.8	1.7	1.3	2.7	4.4	2.6	2.4	3.2
	% Cells in mitosis	0.4	1.7	0.9	0.9	2.2	2.6	2.4	1.6
TD6	Total cells/hr. x 10^6	435.5	497.2	383.4	385.0	317.5	273.8	287.2	286.8
	Abnormal cells/hr. x 10^6	4.2	7.4	8.6	7.0	5.0	3.8	3.9	3.9
	% Abnormal cells	1.0	1.5	2.2	1.8	1.6	1.4	1.4	1.4
	% Cells in mitosis	0.3	1.0	1.5	1.5	0.8	1.2	0.8	0.8

* Abnormal cells are all types listed on pp. 22-24 and include the cells in mitosis whether this is apparently a normal or an abnormal one.

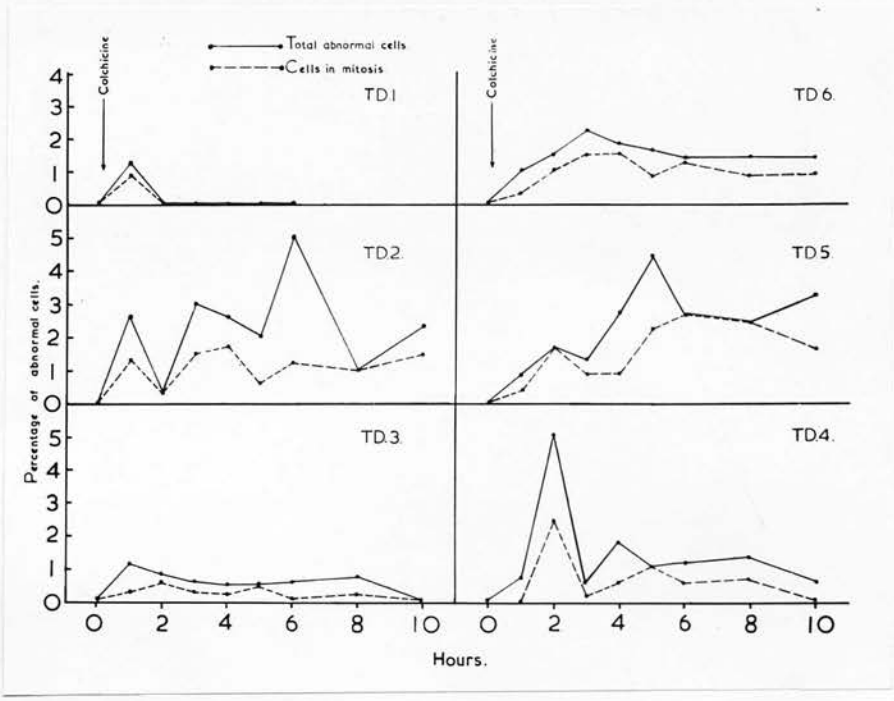

Fig. 7. Percentage of abnormal cells and the cells in mitosis in terms of the total cells of thoracic duct per hour in 6 colchicine-treated rabbits.

of the thoracic duct lymph in the period of 10 hours after the administration of colchicine.

The 'Abnormal' cells

These were seen to appear in the thoracic duct lymph one hour after the intravenous injection of colchicine (but their appearance has been noted as early as 20 minutes following the administration of colchicine in other experiments not reported in the present work). In 4 out of the 6 animals they are still present 10 hours after the injection. Fig. 7. and Table III. show that the abnormal cells form from about 0.2 to 5.0% of the total cells of thoracic duct lymph.

It should, however, be pointed out that not much significance can be attached to the hourly variations in the number of abnormal cells in the thoracic duct lymph, for the percentages are based on relatively small numbers of these cells seen and counted in the haemocytometer chamber.

The various forms of nuclear abnormalities exhibited by the lymphocytes in stained films are illustrated in Figs. 8a to 8k, 9a and 9b. At least 4 types of abnormal cells were seen:

(i) Cells showing mitotic figures. Mitotic figures were only seen in the large lymphocytes and never in the small ones. The mitotic pattern was often that of a metaphase. It will be seen from Figs. 8a to 8e, and 9a to 9b in some of the large cells the chromatin material

Figs. 8a-8k. The cells seen in the thoracic duct lymph of rabbit after the administration of colchicine. May-Grunwald Giemsa x 1000.

Fig. 8a. A large lymphocyte seen in mitosis. The chromosomes appear clumped together and surrounded by a vacuolated cytoplasm.

Fig. 8b. A large lymphocyte seen in mitosis. The chromosomes are isolated and surrounded by a vacuolated cytoplasm. Another large lymphocyte shows a pyknotic nucleus.

Fig. 8c. A large lymphocyte seen in mitosis. The chromosomes are segregated into two groups, one at either pole of the cell. The cytoplasm is vacuolated.

Fig. 8d. Three large lymphocytes seen in mitosis at various stages of metaphase.

Fig. 8e. A large lymphocyte seen in mitosis. The chromosomes are clumped and surrounded by a basophilic cytoplasm. Note the large lymphocyte with two or three nuclei.

Fig. 8f. A small lymphocyte seen with a nucleus undergoing fragmentation and pyknosis.

Figs. 8g. and h. A large and small lymphocyte showing pyknosis of the nucleus.

Figs. 8i and j. A small and large lymphocyte showing nuclear fragmentation (karyolysis).

Fig. 8k. Cytoplasmic fragment with large vacuoles.

Figs. 9a. and b. Large lymphocytes in mitosis as observed in the haemocytometer counting chamber. Brilliant cresyl blue cyanide x 650.

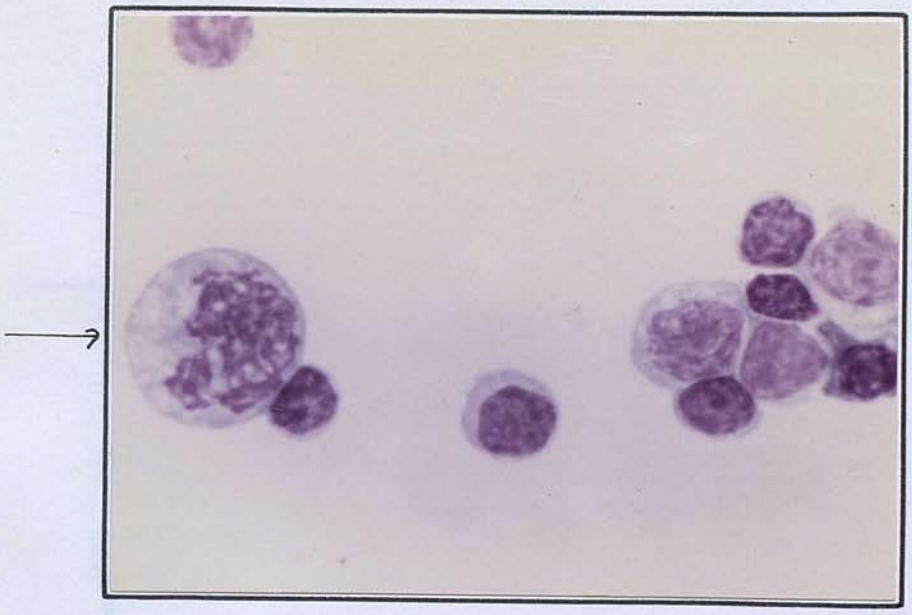

Fig. 8a

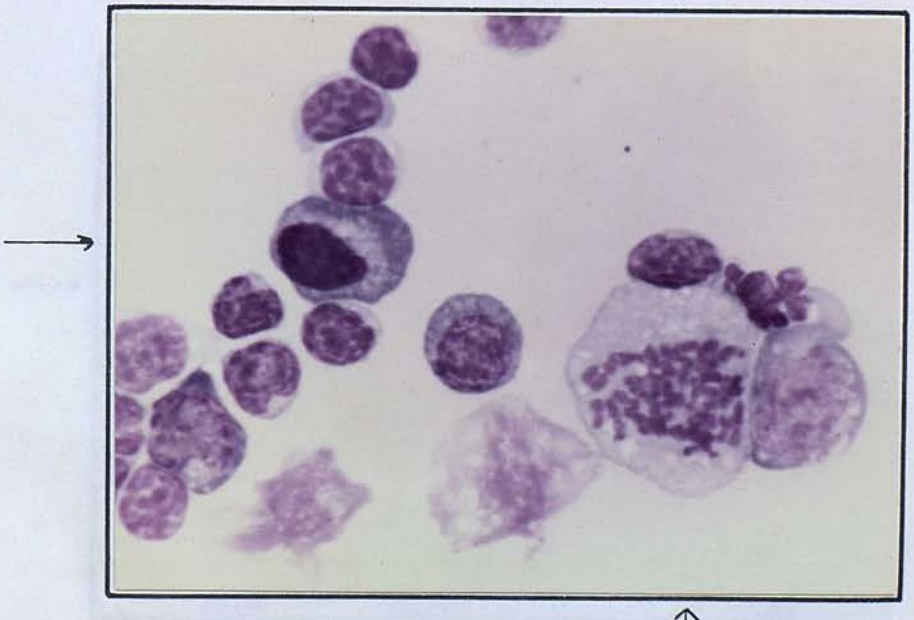

Fig. 8b

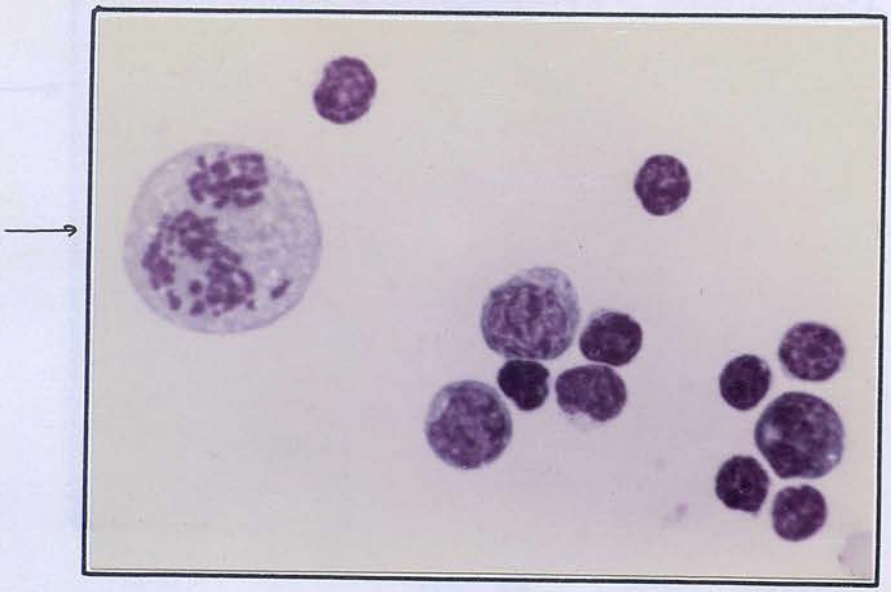

Fig. 8c

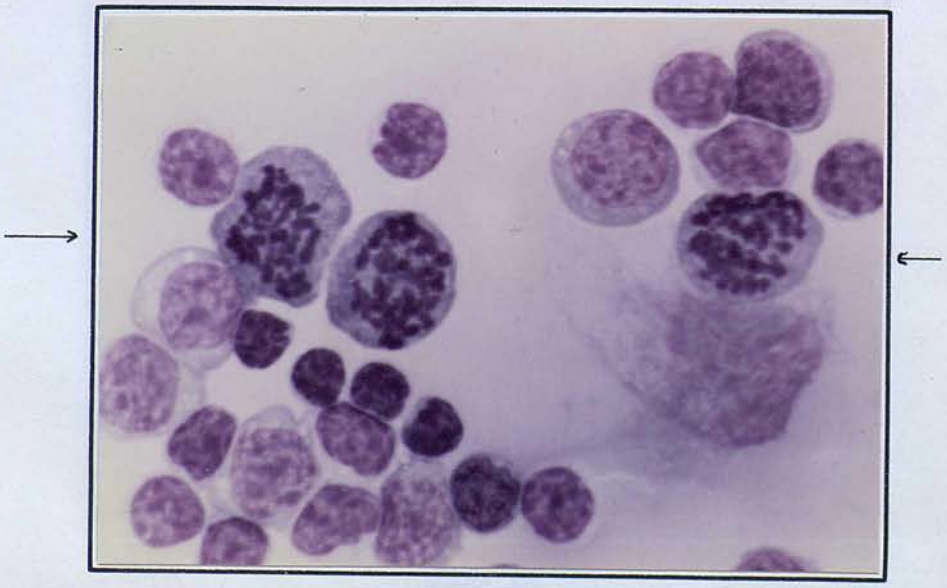

Fig. 8d

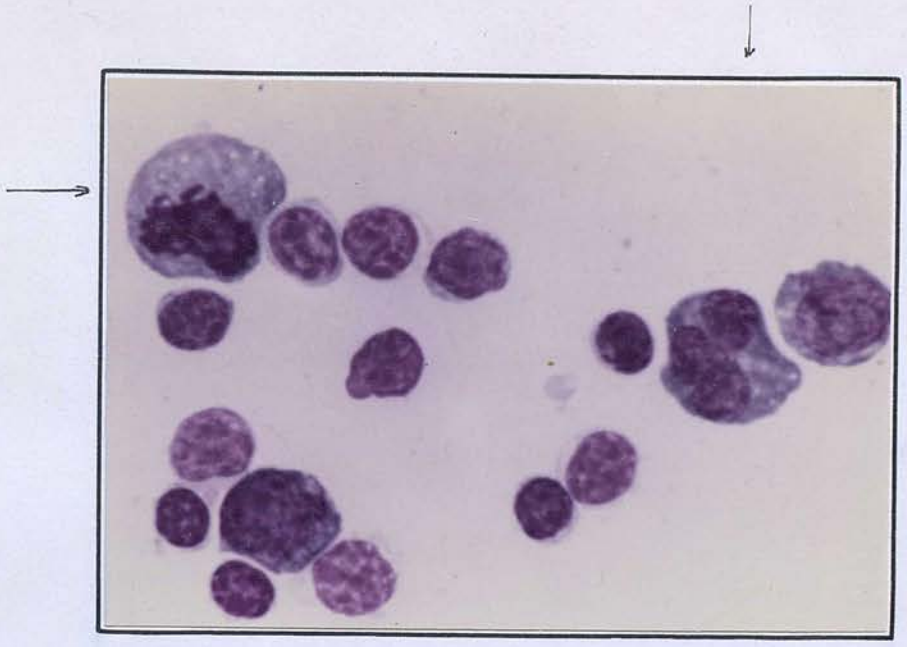

Fig. 8e

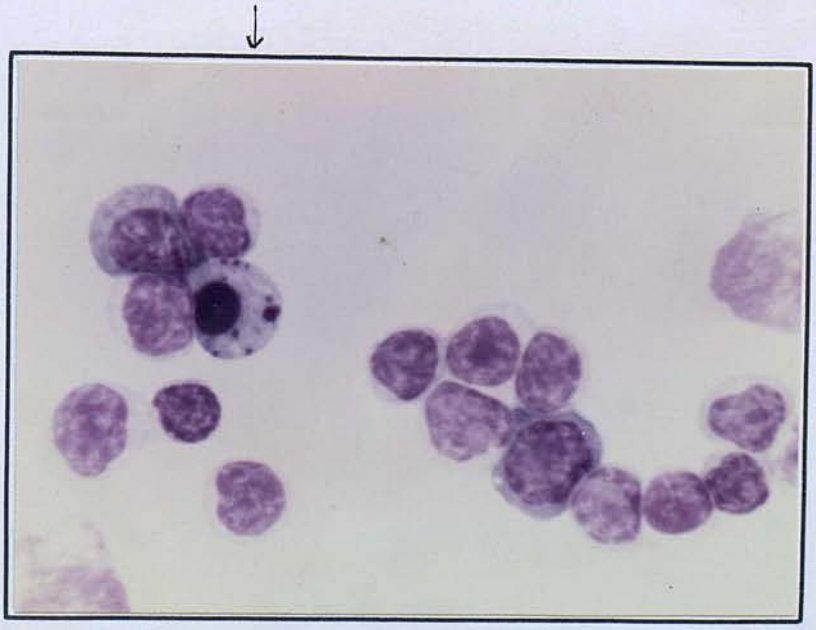

Fig. 8f

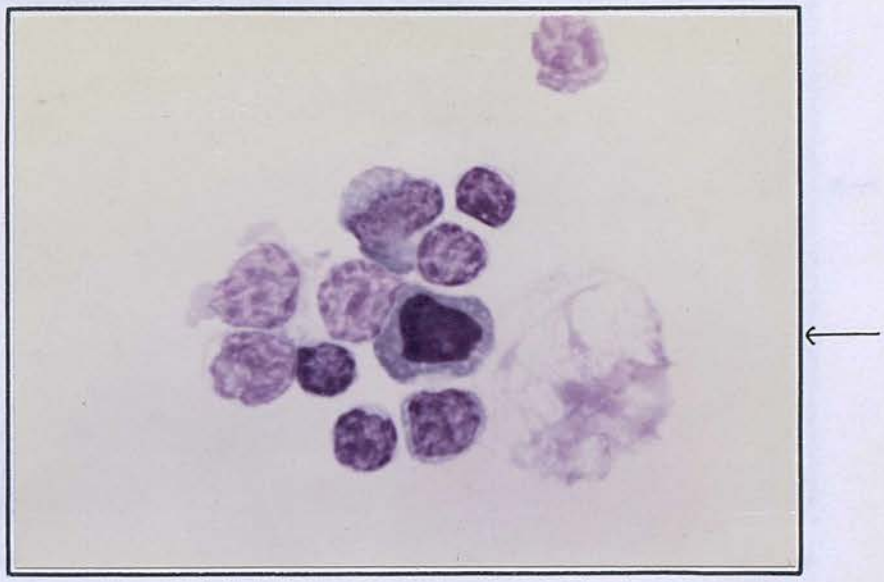

Fig. 8g

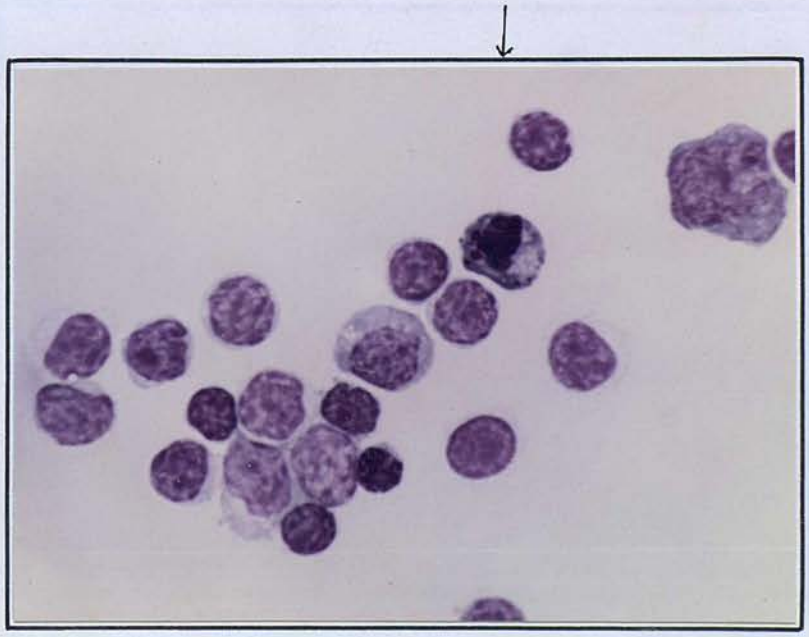

Fig. 8h

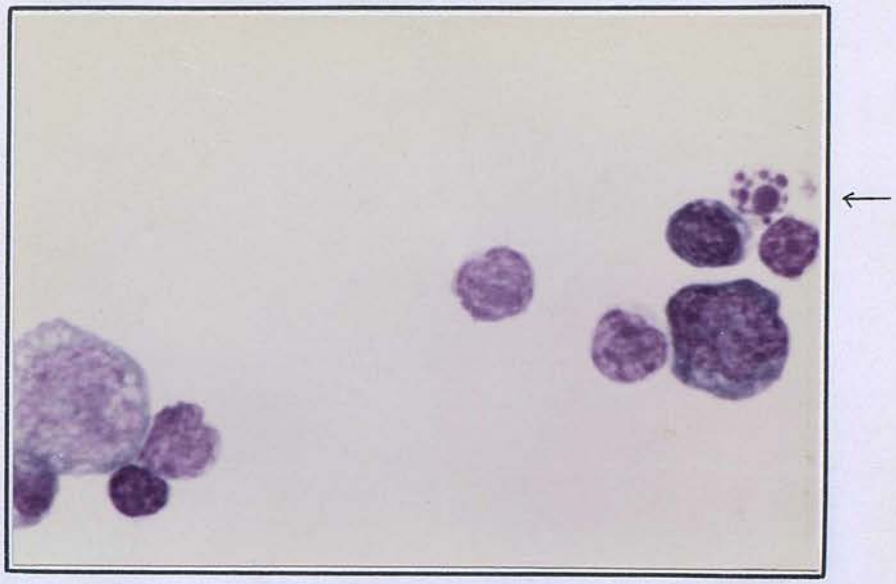

Fig. 8i

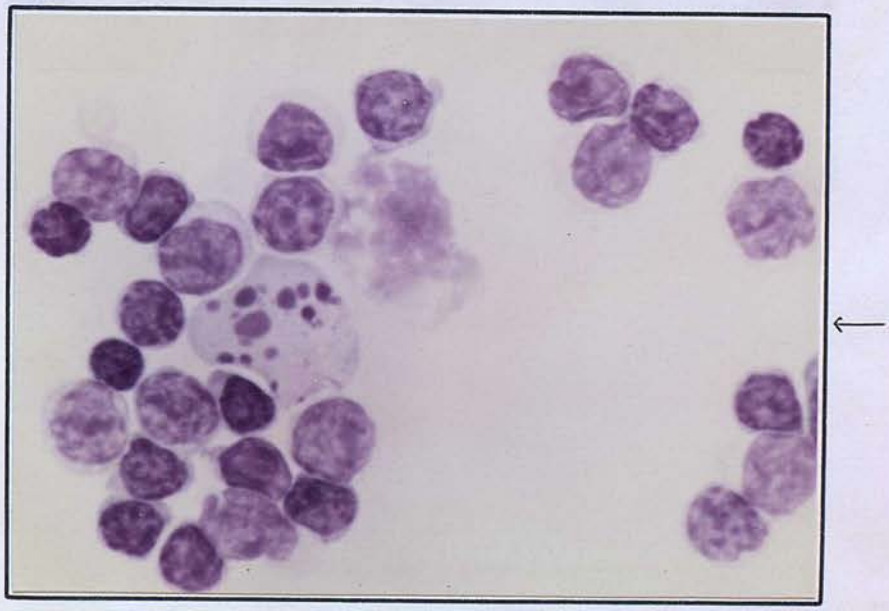

Fig. 8j

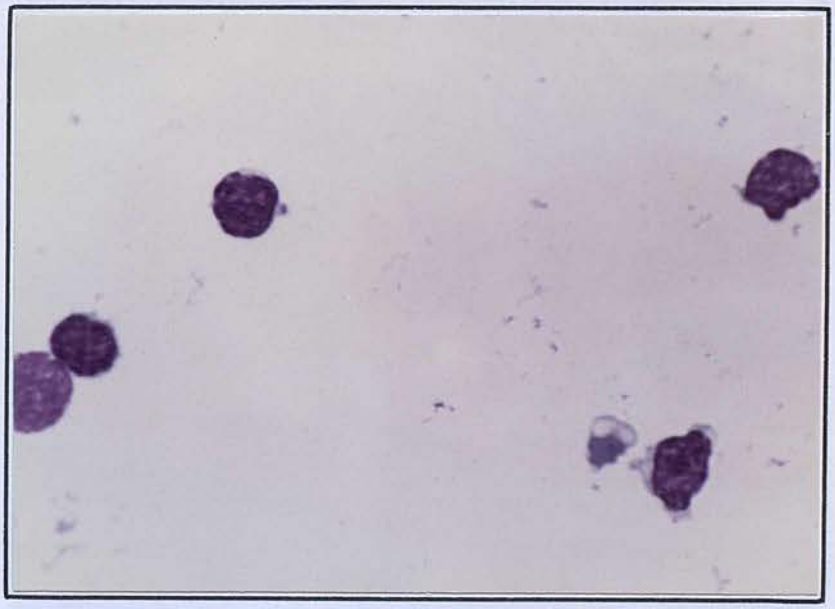

Fig. 8k

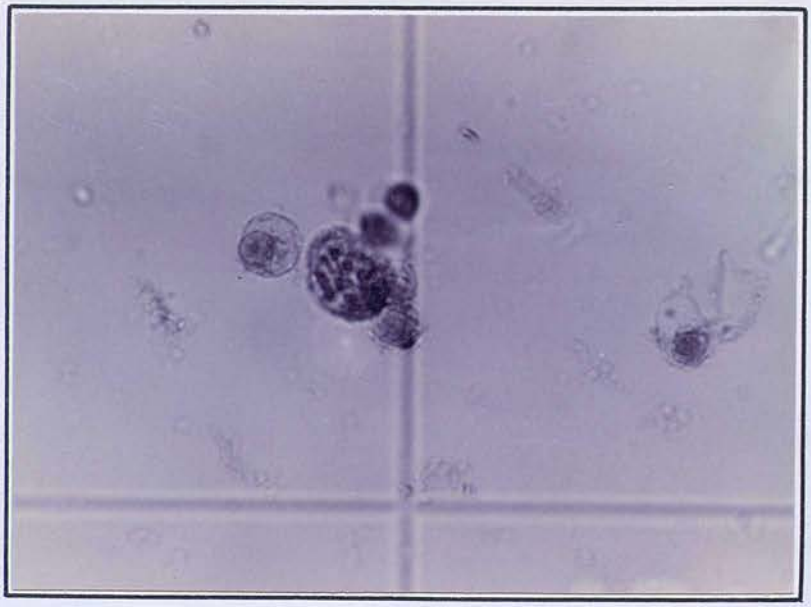

Fig. 9a

Fig. 9b

of the nucleus occurs as a clump in the centre of the cell. Such cells usually show a greater degree of basophilia of the cytoplasm than the other cells with mitotic figures. Other cells were seen in which the chromatin material was broken up into chromosomes which were either scattered throughout the cytoplasm of the cell or arranged around the cell in an orderly fashion. In a few of the cells there was a suggestion that the chromosomes were collected into two groups on either pole of the cell, but in each group the chromosomes can be identified. These various patterns of the metaphase may be considered as corresponding to early, intermediate and late metaphase, even though some of the forms were obviously abnormal. They form at times about 50% of the total abnormal cells and therefore up to 2.5% of the total normal cells coming down the duct (Table III. and Fig. 7.).

(ii) Cells with fragmentation of the nuclei. (Karyolytic nuclei). Some of the cells showed a nucleus which was broken up into two or more fragments. The appearance of these cells is shown in Fig. 8f and 8i to 8j. Fragmented nuclei were not restricted to the large cells alone, but could be seen in both small and large cells. The cytoplasm of these usually showed considerable vacuolation. Up to about 1.0% of the total cells of the thoracic duct lymph were cells of this type.

(iii) Cells with pyknotic nuclei. These forms were

seen mainly in small cells but occasionally in the large lymphocytes. The nucleus in such cells was small and condensed, displaced towards the periphery of the cell, and surrounded by a vacuolated cytoplasm which appeared to be relatively more than that seen in a normal small lymphocyte (Figs. 8f to 8h). These cells form up to about 1.0% of the total cells in the thoracic duct lymph.

(iv) Anuclear cells or fragments. A few cells, or cytoplasmic fragments were seen in which there was no remaining nuclear material. Such fragments were not usually larger than the small lymphocytes present in the smear. These fragments consisted, apparently, of a network of basophilic cytoplasm (~~Figs. 8j and 8k~~). They were present in very small numbers (about 0.5%) of the total cells in the thoracic duct lymph.

It may, however, be pointed out that this classification of the abnormal cells into four types is not a rigid one. A considerable degree of overlap exists among the karyolytic, pyknotic and anuclear types of cells, with one type tending to merge into the other.

3. EFFECT OF COLCHICINE ON THE PERIPHERAL BLOOD IN RABBITS WITH THORACIC DUCT DRAINAGE

a) Effect on the total leucocyte count. Fig. 10 shows the response of the total peripheral blood leucocyte count to an operation in which the thoracic duct was

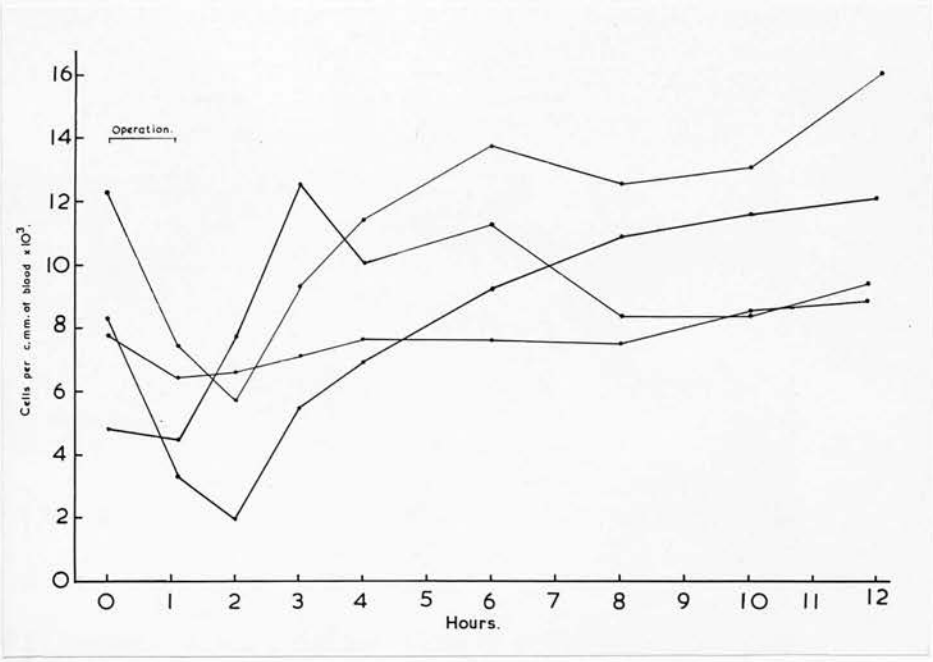

Fig. 10. Total leucocyte counts per cmm.blood in 4 rabbits in which the thoracic duct was drained.

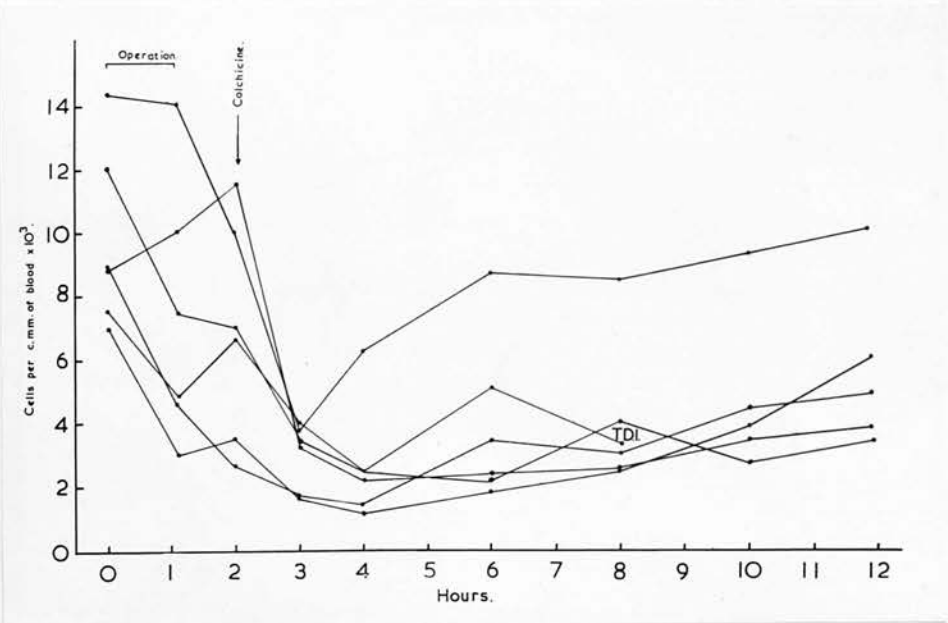

Fig. 11. The effect of colchicine on the total leucocyte counts per cmm. blood in 6 rabbits in which the thoracic duct was drained.

drained in the four control animals. It can be seen that there is a rise in the level of the leucocytes one hour after the operation and a marked leucocytosis which sets in between 2 and 10 hours.

Fig. 11 shows that the level of the total leucocytes per cubic millimetre of blood following the administration of colchicine is greatly depressed during the first hour after colchicine injection. This is consistent with the finding of other workers (Dixon, 1908; Fagraeus, 1953; Harm, 1953). This initial leucopaenia tends to disappear gradually but there is no evidence of any leucocytosis during the period of 10 hours. The above workers found a marked leucocytosis reaching its maximum between 6 and 10 hours. This difference may be due to technical differences or the fact that the other workers used animals which were unanaesthetised and unoperated, whereas the work which is reported here has been carried out on anaesthetised operated animals. Either or both of these two factors, anaesthesia and operation, may be responsible for this difference. As may be seen from the results in the control animals, drainage of the thoracic duct lymph is not by itself the cause of the prolonged leucopaenia seen in the six rabbits, receiving colchicine. It appeared that more study was needed to resolve this problem, and some experiments on these lines are reported as Part II of the present work.

At the present moment it can only be stated that

the effect of colchicine on the blood leucocytes in rabbits in which the thoracic duct is draining is to cause an initial leucopaenia, which decreases very little and tends to persist throughout the period of 10 hours. There is no evidence of any leucocytosis.

b) Effect of colchicine on the blood lymphocyte in animals with thoracic duct drainage. It will be seen in Fig. 12. and Table IV. that, following the operation of thoracic duct drainage there is, in the control animals not receiving colchicine, a pronounced lymphopaenia and that the level of the small lymphocytes in the blood persists at this low level throughout the period of the experiment. It should be noted that, about an hour after the drainage started, the number of large lymphocytes tend to increase gradually.

In the animals receiving colchicine, there is also a similar pronounced lymphopaenia, as may be seen from Fig. 13. and Table V. In some animals, the percentage of large lymphocytes decreases in the first or second hour following colchicine administration, but the response is not regular enough to draw any definite conclusion on this point.

Examination of the blood smears in colchicine-treated rabbits revealed a majority of lymphocytes which looked normal and exhibited no nuclear changes. A few of the small lymphocytes showed nuclear abnormalities of a similar nature to some of those seen in the thoracic duct lymphocytes

Table IV.

Table 4 shows the total number of small and large lymphocytes per cubic millimetre blood and the percentage of large lymphocytes in the normal operated control animals.

Rabbit No.	Variables	Hours							
		Operation							
		0	1	2	3	5	7	9	11
C1	Small lymphocytes	5148	1056	817	724	514	1055	702	760
	Large lymphocytes	390	99	71	57	57	122	138	696
	% Large lymphocytes	7.1	8.6	8.0	7.3	10.0	10.4	16.4	40.9
C2	Small lymphocytes	2784	581	438	280	282	334	236	419
	Large lymphocytes	240	115	125	28	168	84	126	186
	% Large lymphocytes	16.3	16.5	22.2	9.1	37.3	20.1	34.8	30.7
C3	Small lymphocytes	2162	581	394	399	756	500	650	1301
	Large lymphocytes	494	58	120	57	207	188	455	400
	% Large lymphocytes	17.6	8.9	19.6	12.3	21.5	27.3	41.2	23.5
C4	Small lymphocytes	5885	840	880	422	462	926	1270	1219
	Large lymphocytes	585	60	110	109	93	164	230	290
	% Large lymphocytes	9.1	6.6	11.1	20.6	16.7	15.0	15.3	12.7

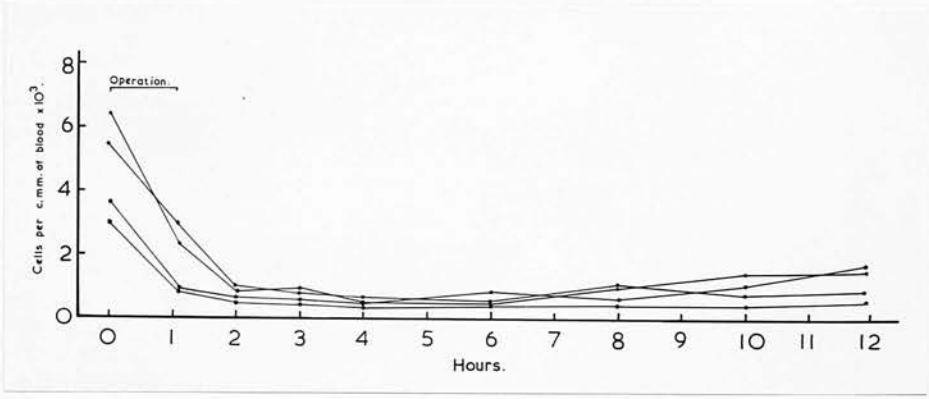

Fig. 12. The number of lymphocytes per cmm. blood in 4 control rabbits in which the thoracic duct was drained.

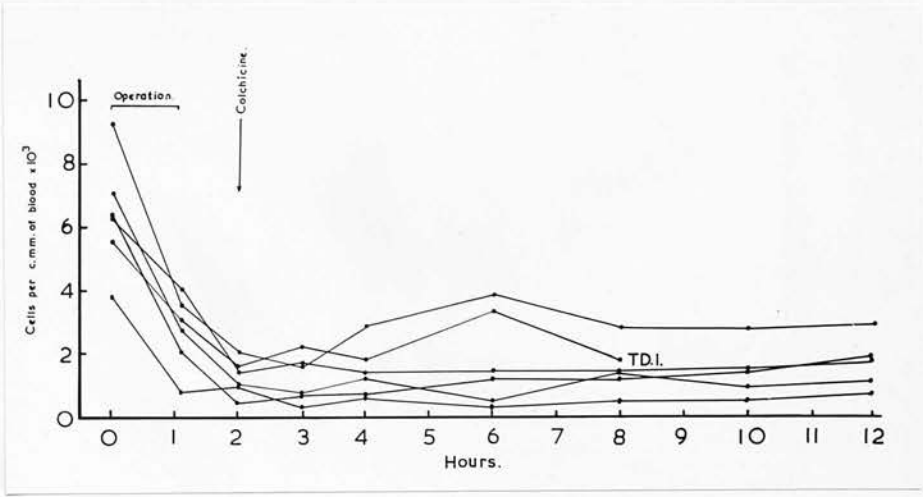

Fig. 13. The effect of colchicine on the number of lymphocytes per cmm. blood in 6 rabbits in which the thoracic duct was drained.

Table V.

Table 5. shows the total number of small and large lymphocytes per cubic millimetre blood and the percentage of large lymphocytes in the colchicine-treated animals.

Rabbit No.	Variables	Hours							
		0	1	2	3	5	7	9	11
		Operation		Colchicine					
TD1	Small lymphocytes	5040	1448	2160	1788	3116	1718	-	-
	Large lymphocytes	450	132	80	62	204	132	-	-
	% Large lymphocytes	8.2	8.3	3.5	3.3	6.1	7.2	-	-
TD2	Small lymphocytes	3340	875	206	588	315	520	515	665
	Large lymphocytes	450	87	33	12	38	50	78	121
	% Large lymphocytes	11.8	9.1	13.4	2.0	10.8	8.6	13.1	15.4
TD3	Small lymphocytes	8530	1920	1592	2699	3600	2468	2337	2200
	Large lymphocytes	720	80	38	286	350	425	558	800
	% Large lymphocytes	7.8	4.0	2.3	9.6	8.9	14.7	19.3	2.6
TD4	Small lymphocytes	5900	1440	1655	1409	1452	1438	1513	1686
	Large lymphocytes	264	57	16	11	48	62	87	114
	% Large lymphocytes	4.3	3.9	1.0	0.9	3.2	4.1	5.2	613
TD5	Small lymphocytes	6598	865	763	1200	522	1472	956	1104
	Large lymphocytes	422	35	17	50	67	108	114	102
	% Large lymphocytes	6.0	3.8	2.2	4.0	11.4	6.9	12.3	8.5
TD 6	Small lymphocytes	5955	430	730	660	1255	1174	1388	1832
	Large lymphocytes	356	55	17	15	107	76	112	168
	% Large lymphocytes	5.6	11.3	2.3	1.9	7.9	6.1	7.4	8.4

Figs. 14a-c. The nuclear abnormalities seen in the blood lymphocytes of rabbits after the administration of colchicine. May-Grunwald Giemsa x 1000.

Fig. 14a. A small lymphocyte showing fragmentation and pyknosis of the nucleus.

Fig. 14b. A large lymphocyte showing vacuolation of the cytoplasm.

Fig. 14c. A small lymphocyte showing pyknosis of the nucleus.

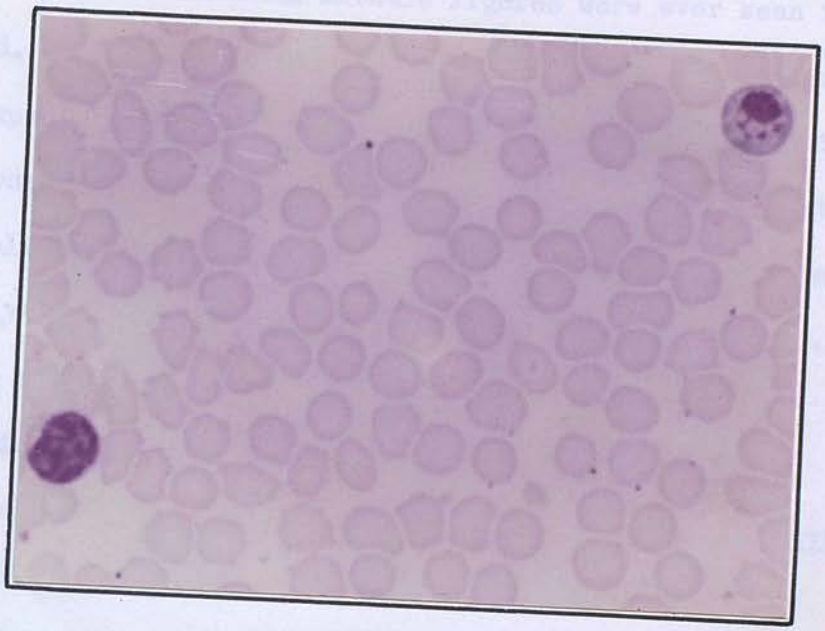

Fig. 14a

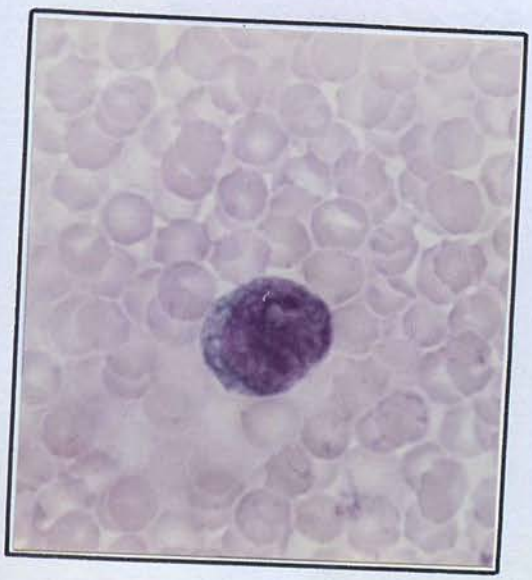

Fig. 14b

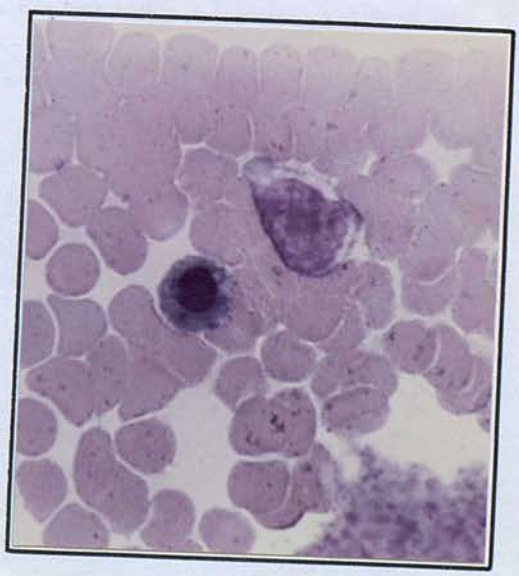

Fig. 14c

of the same animal (Figs. 14a to 14c) except that no cells with normal or abnormal mitotic figures were ever seen in the blood. (It should be noted however that in another series of experiments occasional mitotic figures were seen in the peripheral blood large lymphocytes after the administration of colchicine: a report of this is given later.) Some darkly-stained masses were observed in the blood smears. These may represent smudged nuclei of cells damaged by colchicine.

c) The effect of colchicine on the blood granulocytes. Fig. 15 shows the response of the blood granulocytes to operative drainage of the thoracic duct lymph in the control animals which received no colchicine. There was a granulocytosis in all animals by the third hour after operation and this granulocytosis persisted for the 11 hours of the experiment.

Fig. 16 shows the results obtained in animals receiving colchicine. There was a granulocytopaenia in each animal seen one hour after the administration of colchicine. Even 10 hours after colchicine, the number of granulocytes had only just crept back to pre-operative levels. These changes in the granulocytes are mainly responsible for the leucopaenia shown in Figs 15 and 16. This is also illustrated in Figs. 17a and 17b. which show the changes in total leucocytes, lymphocytes and granulocytes in two animals.

It is apparent that colchicine is, in the conditions

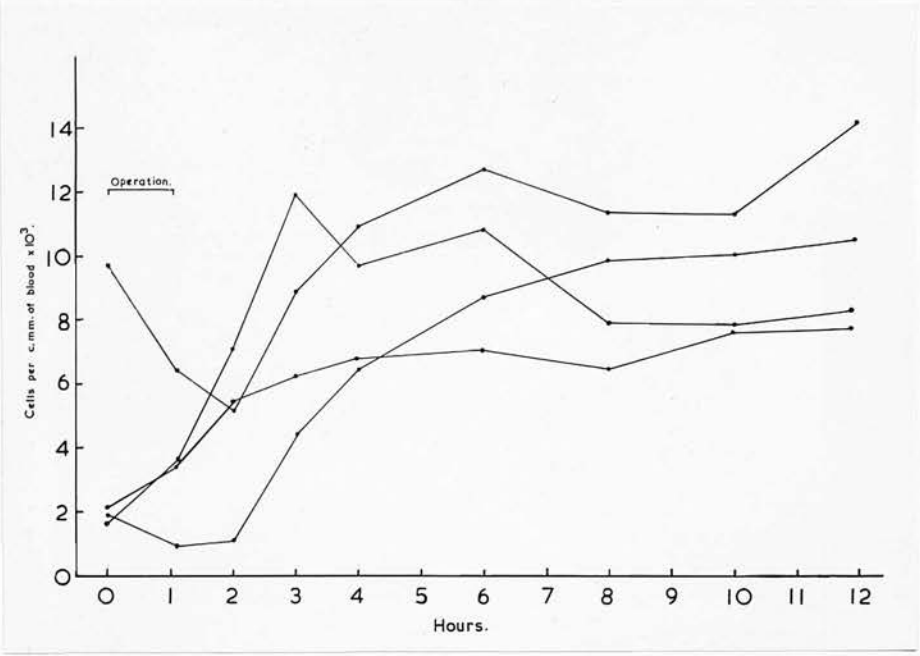

Fig. 15. The number of granulocytes per cmm. blood in 4 control rabbits in which the thoracic duct was drained.

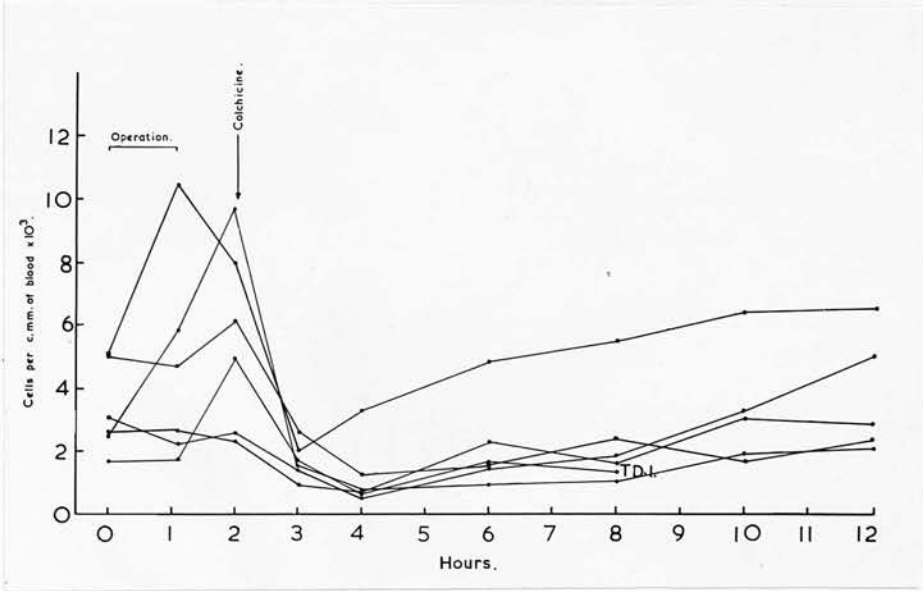

Fig. 16. The effect of colchicine on the granulocytes per cmm. blood in 6 rabbits in which the thoracic duct was drained.

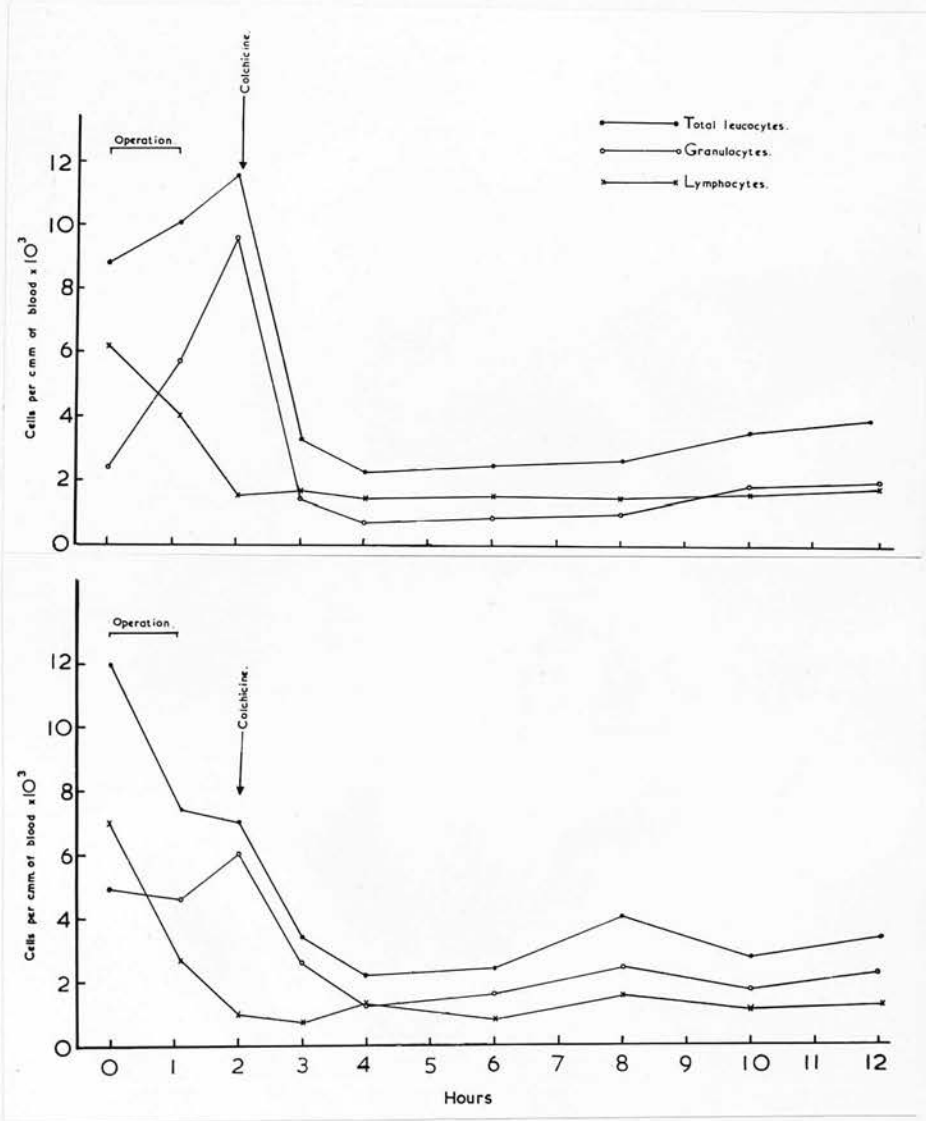

Fig. 17a and 17b. The effect of colchicine on the total leucocytes, the lymphocytes and the granulocytes per cmm. of blood in 2 rabbits in which the thoracic duct was drained.

c') Effect of colchicine on the monocytes of the peripheral blood. In the majority of the experiments, the blood monocytes after the injection of colchicine decreased considerably from the normal level of about from 250 to 25 cells per cubic millimetre of blood. In some smears, no monocytes could be seen 2 to 3 hours after colchicine administration. They reappeared usually about 8 hours later. A similar effect was also seen in those rabbits which had thoracic duct drainage without colchicine.

It may be mentioned here that in other experiments anaesthesia alone or with operation interference, caused the blood monocytes to fall to very low levels. The effect of colchicine cannot be considered to be a specific reaction, for a wide variety of stimuli cause the same response.

of the experiment, acting to inhibit or delay the granulocytosis that accompanies the operative interference of thoracic duct drainage. This problem is investigated further in Part II of the present work.

The above changes refer to the most common type of granulocyte present in the peripheral blood of rabbits - a cell equivalent to the neutrophil polymorphonuclear leucocyte of the human, which is sometimes called in rabbits the pseudoeosinophil. There was no suggestion of any marked change in the number or character of the basophil or the true eosinophil polymorphonuclear leucocyte in the conditions of these experiments.

The character of some of the granulocytes as seen in May-Grunwald Giemsa stained films was altered in the colchicine-treated animals. The cells tended to rupture easily, and these cells showed swollen poorly stained nuclei, and, in some, the granules were extruded. In two out of the six rabbits receiving colchicine some of the granulocytes had rounder, more globular and much denser nuclei than was seen normally.

d) The effect of colchicine on the erythrocytes of the peripheral blood. It may be noted that there were changes in the circulating erythrocytes in the rabbits which had received colchicine, the most obvious change being the appearance of normoblasts (Fig. 18.). A few typical normoblasts were usually seen in the peripheral

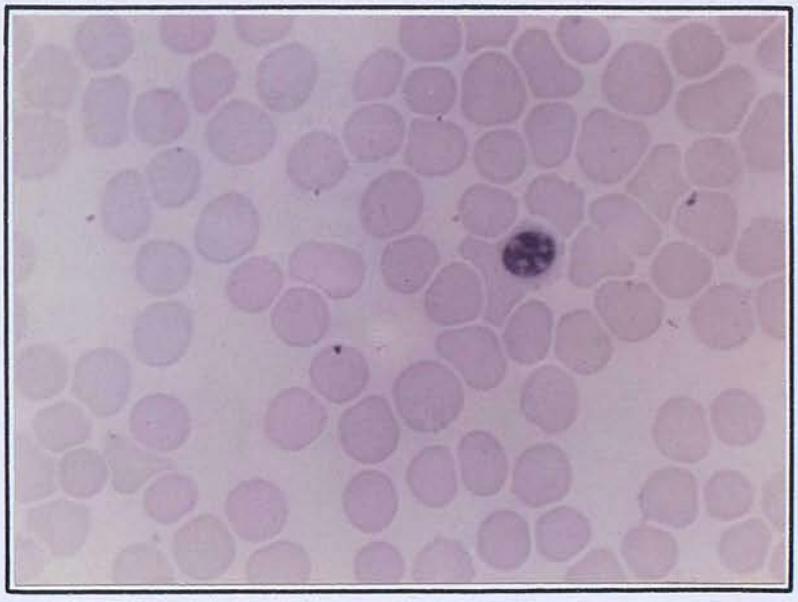

Fig. 18. A normoblast in the peripheral blood 4 hours after the administration of colchicine to a rabbit. Blood smear. May-Grunwald Giemsa x 1000.

Table VI.

Table VI. shows the weight of spleen in control and the colchicine-treated animals. In both cases the thoracic duct had been drained for 11 hours.

Colchicine Treated		Normal Controls	
Rabbit No.	Spleen wt.mg./kg. body weight	Rabbit No.	Spleen wt. mg./kg. body weight
TD1	223.2	C1	439.4
TD2	232.2	C2	327.3
TD3	134.0	C3	290.4
TD4	208.3	C4	455.1
TD5	318.6		
TD6	225.9		
	223.2±58.7		378.1±81.3

blood two hours after the administration of colchicine. Their number increased until between the 6th and 8th hour there were from about six to eight normoblasts for every 200 white cells counted. Some of the red blood cells exhibited polychromasia and in others a basophilic reticulum could be seen. No red blood cell counts or haemoglobin estimations were made in these experiments.

4. GROSS AND MICROSCOPICAL EXAMINATION OF THE SPLEEN

a) Gross findings. The spleen weights in the control rabbits and in those receiving colchicine are recorded in Table VI. The spleen was weighed eleven hours after the thoracic duct had been cut and, in those receiving colchicine, ten hours after the injection of the alkaloid. It can be seen that the spleens are smaller in the colchicine-treated rabbits than in the controls.

b) Microscopic findings. An occasional lymphocyte in mitosis was seen in imprints from the colchicine-treated rabbits, but none was ever seen in the imprints of control animals.

The most striking feature of the sections of spleen was the presence of large amount of nuclear debris in the red pulp and this was not seen in normal spleen sections (Figs. 19. and 20.). The white pulp in no way appeared to differ from that of the normal spleens.

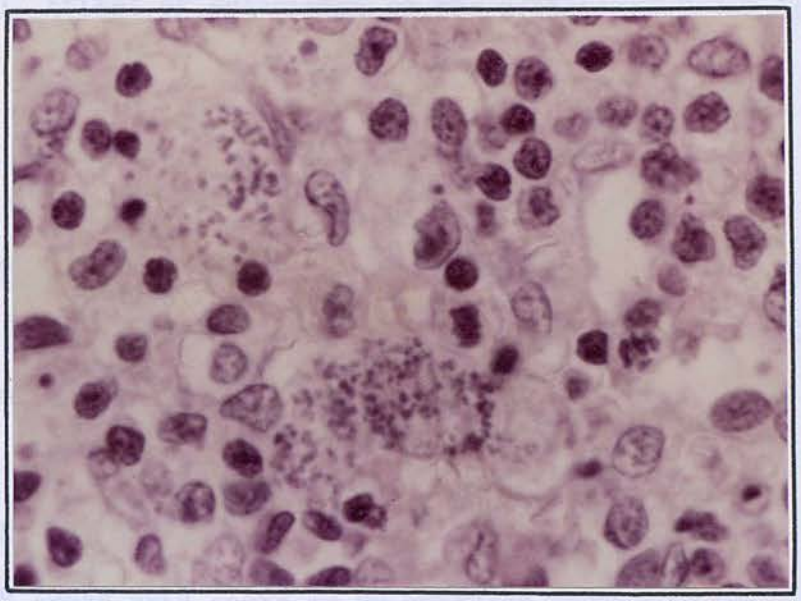

Fig. 19. Section of spleen from a rabbit taken 10 hours after the injection of colchicine and 11 hours after lymph drainage. Nuclear debris can be seen in the red pulp. Haemotoxylin and cosin x 1000.

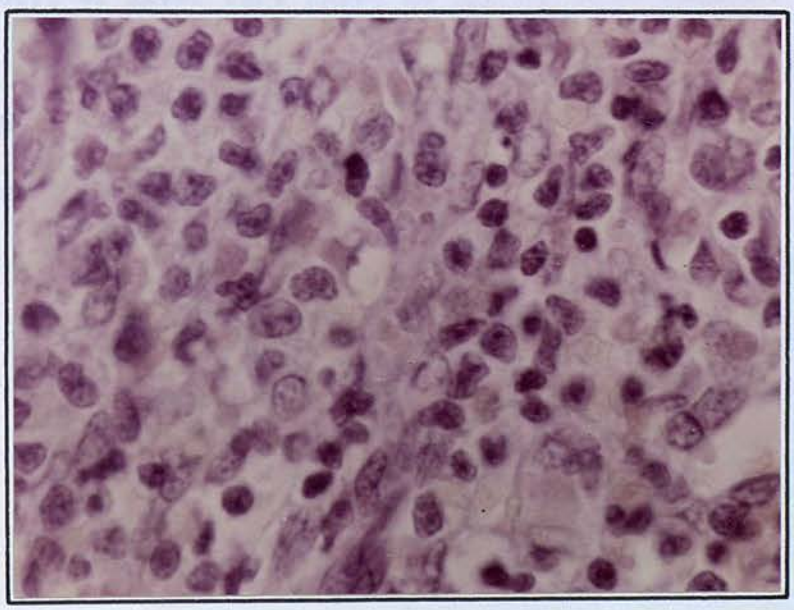

Fig. 20. Section of spleen from a rabbit taken 11 hours after lymph drainage alone. No nuclear debris can be seen. Haemotoxylin and cosin x 1000.

Part II: Effect of colchicine on the peripheral blood leucocytes

EXPERIMENTAL METHODS

The methods used in these experiments are those given in Part I. (Pp. 4-16). In addition the following histological methods were used.

Histology of the lung

The lungs were fixed in an inflated condition by injecting Susa fixative into the trachea (Carleton, 1957). Paraffin wax sections were cut at about 5μ and stained with haemotoxylin and eosin.

Histology of the liver

Pieces of liver were fixed in 10% buffered formol-saline, washed, dehydrated, cleared and embedded in paraffin wax. Sections were cut at about 5μ and stained with haemotoxylin and eosin.

Part II: Effect of colchicine on the peripheral blood leucocytes

RESULTS

The results of a series of experiments which were done to study the effect of colchicine on the blood leucocyte and in particular, on the blood granulocyte, are given below, and are shown in the accompanying Figs. 21-33 and Tables VII.-XI. These experiments were performed with a view to finding the cause of the persisting granulocytopaenia which was observed after the intravenous injection of colchicine into the anaesthetised rabbits with lymph drainage (Part I. of the present work).

It has been reported by Dixon and Malden (1908) that in rabbits, the granulocytopaenia following the subcutaneous injection of colchicine disappears gradually after from 1 to 2 hours, resulting in a granulocytosis which reaches its peak from 6 to 10 hours. They used the subcutaneous route for the administration of colchicine in unanaesthetized animals and it was thought that our results might be due to the different route used for the administration of colchicine, or to the operative interference. Therefore, the observation of the previous workers was re-investigated using colchicine intravenously in unanaesthetized unoperated rabbits.

1. EFFECT OF COLCHICINE ON THE BLOOD GRANULOCYTES

a) Effect of colchicine on the blood granulocytes in unanaesthetised normal rabbits. Ten rabbits were used.

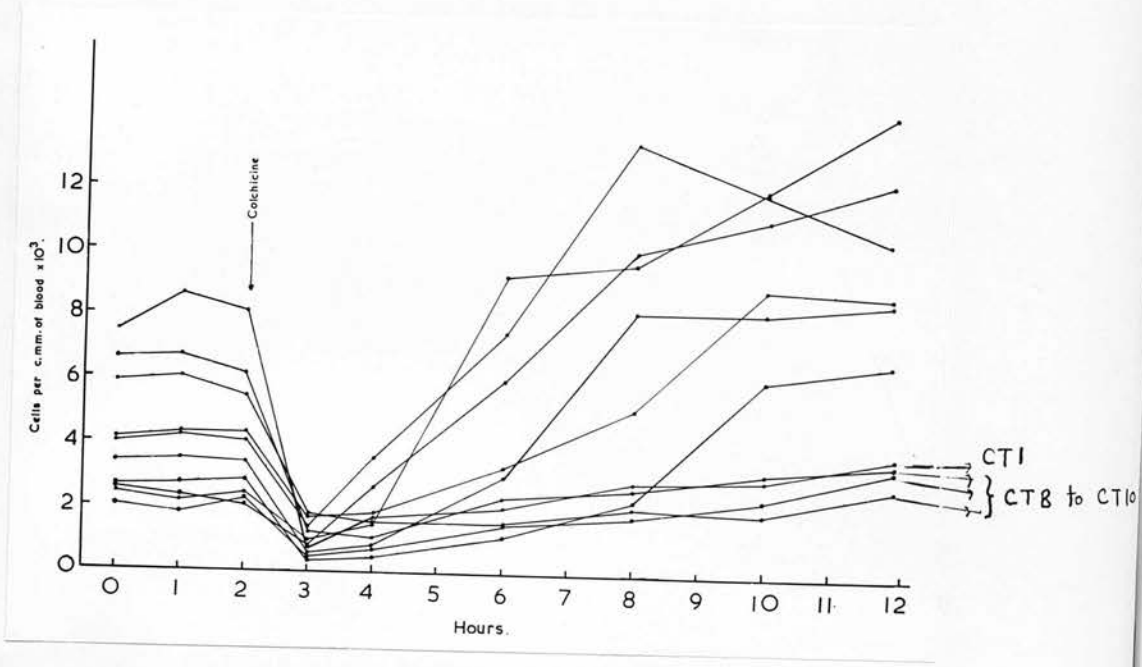

Fig. 21. The effect of colchicine on the number of granulocytes per cmm. blood in 10 unanaesthetised rabbits.

Three samples of blood were removed from the ear vein at 0, 1 and 2 hours, then 2.5mg./kg. body weight of colchicine was injected into the other ear vein, and samples of blood for total and differential counts were taken for a period of 10 hours at hourly and two hourly intervals.

The results of these experiments are shown in Fig. 21. It may be seen that there is a pronounced fall in the level of the blood granulocytes 1 hour after the injection. After this initial granulocytopaenia in 6 of the rabbits, the level of blood granulocytes rises and continues to rise for some hours, exceeding the basal pre-injection levels.

In rabbit No. CT1 the rise after the initial depression is slow and basal levels are not reached until about 6 hours after the injection of colchicine. In the remaining 3 animals, Nos. CT8 to CT10, there is no evidence of any granulocytosis during the period of the observations. It may however be noted that in these 3 animals the basal levels of the granulocytes before the injection of colchicine are higher than those of the other 7 animals, contributing from about 68 to 85% of the total leucocytes as against the normal levels of from 30 to 50%, which were seen in the other 7 rabbits. Furthermore, these animals were experimented upon simultaneously on the same day and belonged to the same fresh stock. It was therefore thought that this variation in the response to colchicine may be due to the effect of a concurrent infection which the rabbits might have had. A

post-mortem examination on these 3 animals was therefore made and it was seen that the liver was covered with small, round yellowish-white patches varying in size from pin-points to about 4mm. in diameter, suggestive of Coccidiosis.

In view of the above, the results of the experiments on these 3 pathological animals are not strictly comparable with the other normal rabbits. The results in the 7 normal rabbits are in agreement with those of the previous workers. The cause of the persisting granulocytopaenia in the anaesthetised, operated animals with lymph drainage appears to be not accounted for by the different route of administration of colchicine.

Further experiments were therefore performed in order to investigate possible factors responsible for the granulocytopaenia in the rabbits with thoracic duct drainage.

b) The effect of colchicine on the blood granulocytes in the sham-operated rabbits. Four rabbits were used. A sample of blood was taken at 0 hours. An operation was done on the right side of the neck, in an identical manner to the operation for exposure of the thoracic duct. Blood samples were taken on completion of the operation and the following hour. Colchicine was then injected intravenously and thereafter blood samples obtained for total and differential leucocyte counts, at hourly or two hourly intervals, over a period of 10 hours.

Fig. 22 shows the results of these experiments. It will

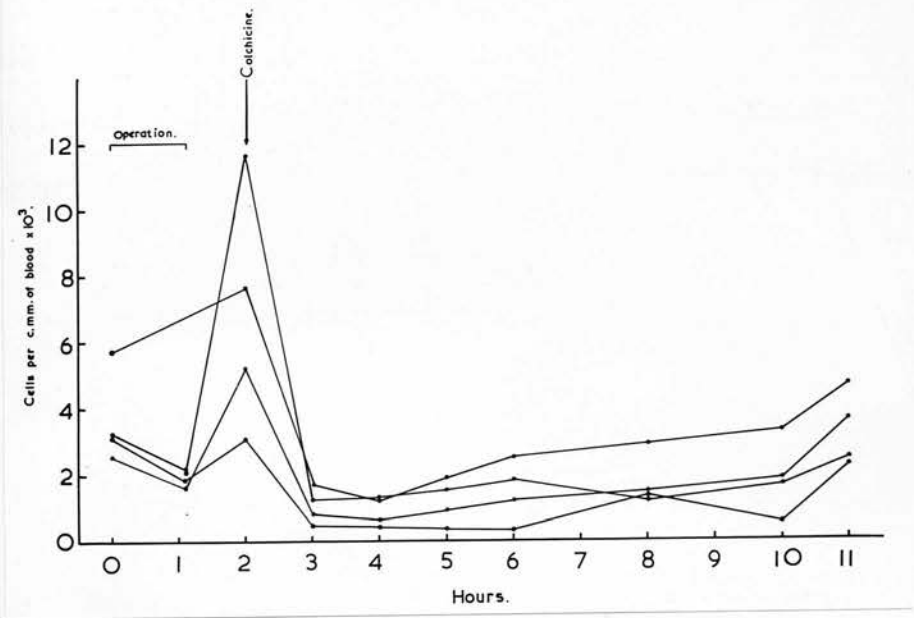

Fig. 22. The effect of colchicine on the number of granulocytes per cmm. of blood in 4 sham-operated rabbits.

be seen that an hour after the operation there is a definite granulocytosis at which time colchicine was injected. Following the administration of colchicine a profound granulocytopaenia occurs within an hour, and this persists, for the experimental period, with very little or no recovery. There is no indication of a granulocytosis at 6 to 8 hours.

The results of this experiment therefore suggest that the drainage of lymph cannot be the causative factor for the persisting granulocytopaenia in the group of 6 rabbits with lymph drainage, because the granulocytopaenia occurs even in these animals in which there was no interference with the flow of lymph. This is further supported by the experiments on the 4 control rabbits with lymph drainage not receiving colchicine (p. 27), in which a marked and persisting granulocytosis occured during the experimental period, in spite of the lymph being drained.

c) Effect of the operation on the blood granulocytes. In 2 rabbits, a sham operation was done on the right side of the neck as described above, but in these animals no colchicine was given. Blood samples were removed as in other experiments.

It will be seen from Fig. 23 that one hour after the operation a profound granulocytosis occurs.

The operation itself is obviously not the cause of the persisting granulocytopaenia in the anaesthetised and operated animals receiving colchicine .

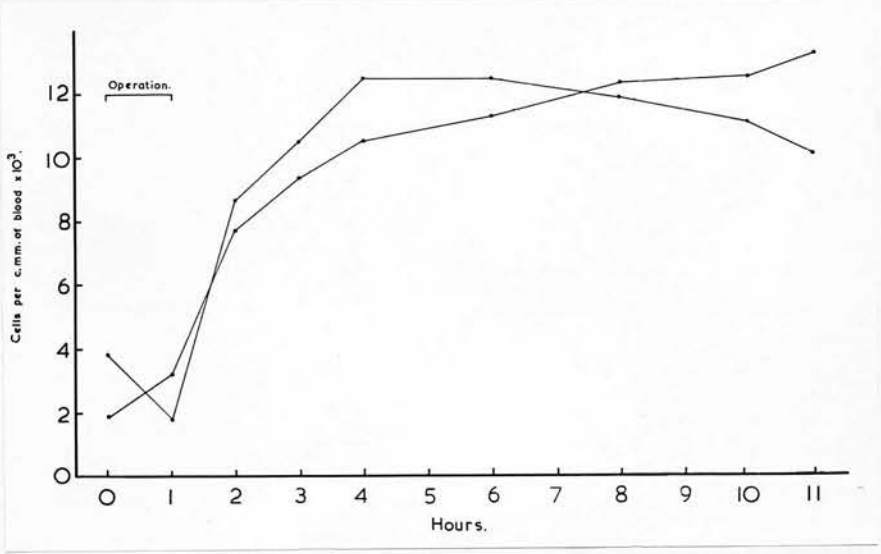

Fig. 23. The effect of sham-operation alone (without colchicine) on the number of granulocytes per cmm. of blood in 2 rabbits.

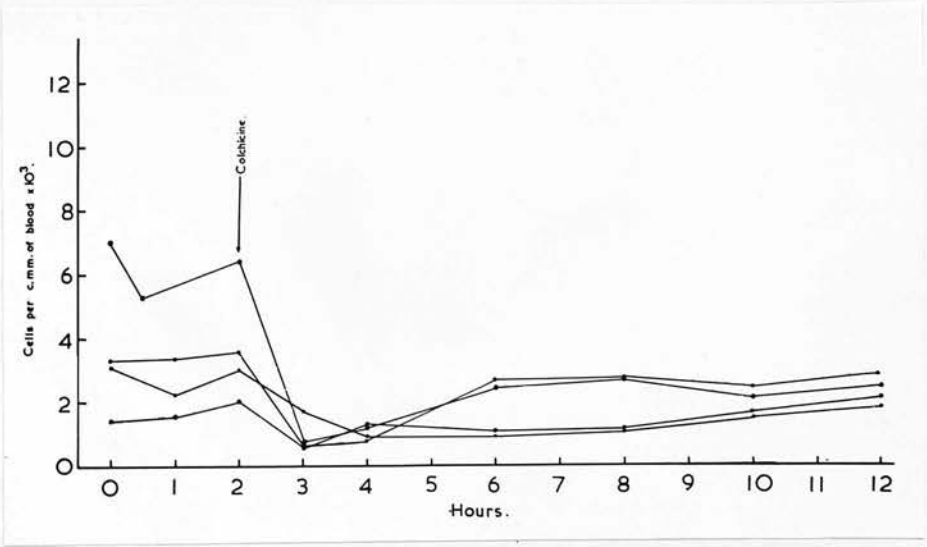

Fig. 24. The effect of colchicine on the number of granulocytes per cmm. of blood in 4 anaesthetised rabbits.

d) Effect of colchicine on the blood granulocytes in anaesthetised, unoperated rabbits. Four rabbits were used. Hourly samples of blood were taken up to 2 hours and the colchicine was injected immediately and sample of blood were taken as before.

Fig. 24 shows that there was a granulocytopaenia 1 hour after the injection of colchicine which persisted throughout the experimental period with little recovery.

The results of these experiments therefore suggest the possibility of the anaesthetic acting in conjunction with the colchicine to produce the observed deviation in response to colchicine from the normal pattern in the anaesthetised and operated animals. The following groups of experiments appear to lend support to this view.

e) Effect of anaesthesia alone on the blood granulocytes of rabbits. Four rabbits were used. Samples of blood were obtained as in the other experiments.

Fig. 25 shows the results of these experiments. It may be seen that within from 2 to 3 hours after the anaesthesia there is a marked granulocytosis which persists throughout the experiment.

Anaesthesia by itself, therefore, produces a granulocytosis but in the colchicine-treated animal as noted above, it tends to produce a severe persisting granulocytopaenia.

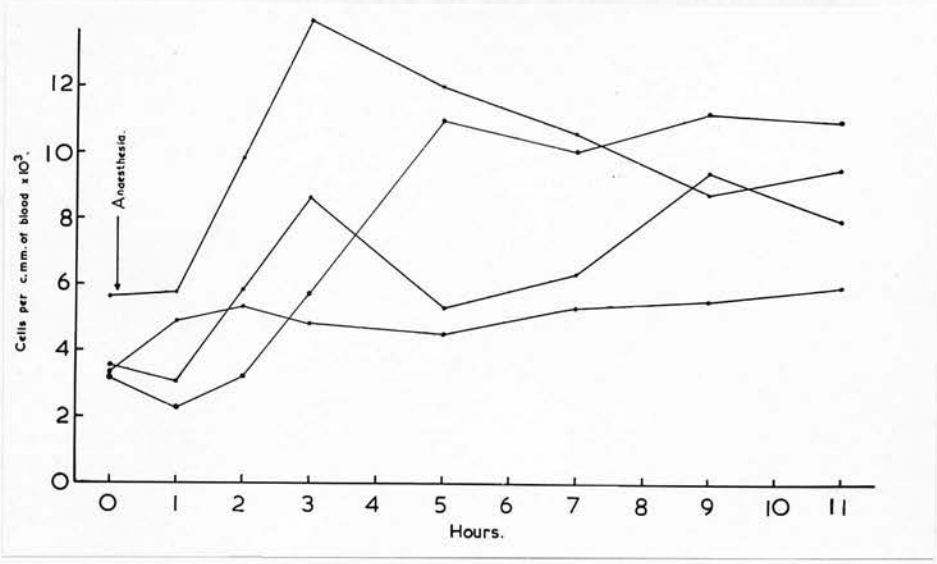

Fig. 25. The effect of anaesthesia alone on the number of granulocytes per cmm. of blood in 4 rabbits.

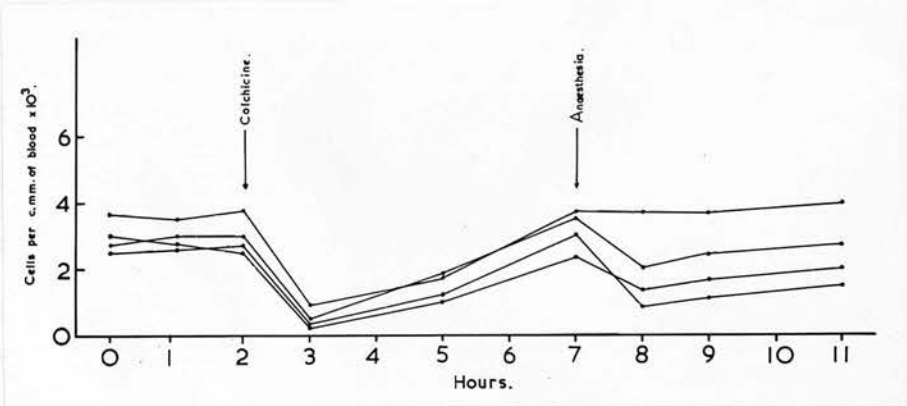

Fig. 26. The effect of colchicine followed 5 hours later by anaesthesia on the number of granulocytes per cmm. of blood in 4 rabbits.

f) Effect of anaesthesia on the blood granulocytes, five hours after the administration of colchicine. A group of 4 rabbits was used. Samples of blood were taken before and after the injection at the usual intervals. At the 5th hour the rabbits were anaesthetised.

The results of these experiments are shown in Fig. 26. It will be seen that before the administration of the anaesthetic, the level of the granulocytes had recovered from the initial depression caused by the injection of colchicine. On the injection of anaesthetic (a procedure which normally gives rise to a granulocytosis, Fig. 25), there is either a fall in the level of the granulocytes or the granulocytes tend to remain at the same level.

From the results of all these experiments it appears that anaesthesia in the presence of colchicine tends to abolish or delay the onset of the granulocytosis which usually follows from 6 to 8 hours after the administration of colchicine in the normal non-anaesthetised rabbits. Lymph drainage, the operative procedure, and the anaesthesia by themselves do not cause a granulocytopaenia but on the contrary produce a granulocytosis.

g) Microscopic examination of blood smears. Examination of the smears of samples of blood obtained after colchicine injection in the different groups of experiments showed the

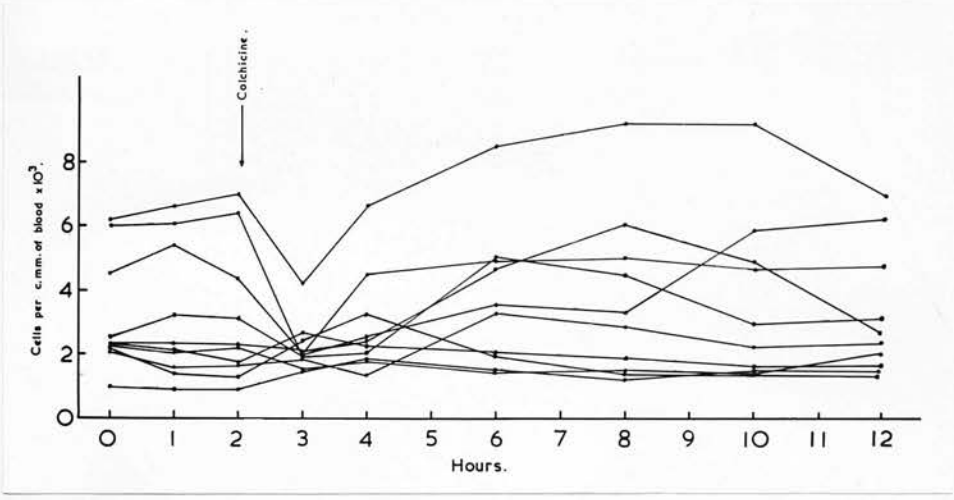

Fig. 27. The effect of colchicine on the number of lymphocytes per cmm. of blood in 10 unanaesthetised rabbits.

same effects on the neutrophils and the cells of the erythrocyte series as reported in Part I. (Pp. 28-29).

2. THE EFFECT OF COLCHICINE AND VARIOUS OPERATIVE PROCEDURES ON THE BLOOD LYMPHOCYTES

The level of blood lymphocytes, as well as the level of blood granulocytes, was estimated in each of the experimental groups described above.

a) The effect on the number of blood lymphocytes. It can be seen from Fig. 27 and Table VII that in the unanaesthetised rabbits, a fall in the blood lymphocytes is sometimes seen 1 hour after the injection of colchicine. This lymphopaenia appeared in rabbits in which the basal level of blood lymphocytes was high; with low levels, colchicine appears to have little or no effect on the number of lymphocytes. Complete recovery from the lymphopaenia usually takes place quickly.

Figs. 28 and 29 and Tables VIII and IX show the effect of colchicine on the number of lymphocytes in the sham-operated and in the anaesthetised rabbits. It will be seen that the level of lymphocytes falls before the injection of colchicine due either to the operation or the anaesthesia or to both, and that the administration of colchicine has little or no effect on this lymphopaenia.

From Figs. 30 and 31 and Tables X and XIa, it can be seen that anaesthesia alone or sham-operation in the anaesthetised animal produces, without the administration of colchicine, a

Table VII

Table VII. shows the number of lymphocytes per cmm. of blood before and after colchicine injection in a group of 10 unanaesthetised rabbits, and the absolute numbers of the small and the large lymphocytes in 6 of the same animals.

Rabbit No.	Variables	Hours								
				Colchicine ↓						
		0	1	2	3	4	6	8	10	12
CT1	Total lymphocytes/cmm.	6,000	6,144	6,450	1,911	2,553	3,564	3,389	5,922	6,278
	Small lymphocytes/cmm.	5,450	5,548	5,800	1,833	2,449	3,366	2,927	5,217	5,548
	Large lymphocytes/cmm.	550	596	650	73	104	198	462	705	730
CT2	Total lymphocytes/cmm.	2,340	2,340	2,250	2,015	2,450	4,680	6,140	5,950	3,672
	Small lymphocytes/cmm.	2.07o	2.08o	2,000	1,893	2,334	4,446	5,900	5,712	3,468
	Large lymphocytes/cmm.	270	260	250	122	116	234	240	238	204
CT3	Total lymphocytes/cmm.	2,180	2,140	2,210	1,576	1,850	1,472	1,518	1,456	2,141
	Small lymphocytes/cmm.	1,900	1,890	1,950	o,478	1,702	1,329	1,320	1,248	1,819
	Large lymphocytes/cmm.	280	252	260	98	148	143	198	208	322
CT4	Total lymphocytes/cmm.	2,500	3,250	3,100	1,850	2,080	5,000	4,500	3,000	3,200
CT5	Total lymphocytes/cmm.	2,090	1,599	1,600	1,810	1,325	3,290	2,850	2,230	2,440
CT6	Total lymphocytes/cmm.	6,250	6,625	7,000	4,260	6,600	8,500	9,250	9,250	6,225
CT7	Total lymphocytes/cmm.	4,550	5,400	4,390	o,979	4,500	4,990	5,000	4,650	4,850

Table VII (contd.)

Rabbit No.	Variables	Hours								
				Colchicine ↓						
		0	1	2	3	4	6	8	10	12
CT8	Total lymphocytes/cmm.	1,020	865	850	1,530	1,965	1,435	1,200	1,460	1,110
	Small lymphocytes/cmm.	807	723	672	1,484	1,893	1,365	1,120	1,370	1,016
	Large lymphocytes/cmm.	213	142	178	46	72	70	80	90	94
CT9	Total lymphocytes/cmm.	2,360	2,290	1,790	2,720	2,220	2,060	1,900	1,655	1,630
	Small lymphocytes/cmm.	2,203	2,058	1,645	2,675	2,105	1,952	1,819	1,513	1,498
	Large lymphocytes/cmm.	157	232	145	45	115	108	81	142	132
CT10	Total lymphocytes/cmm.	2,200	1,485	1,355	2,420	3,230	1,900	1,400	1,490	1,310
	Small lymphocytes/cmm.	1,936	1,238	1,055	2,385	3,186	1,857	1,280	1,400	1,220
	Large lymphocytes/cmm.	264	247	300	35	44	43	120	90	90

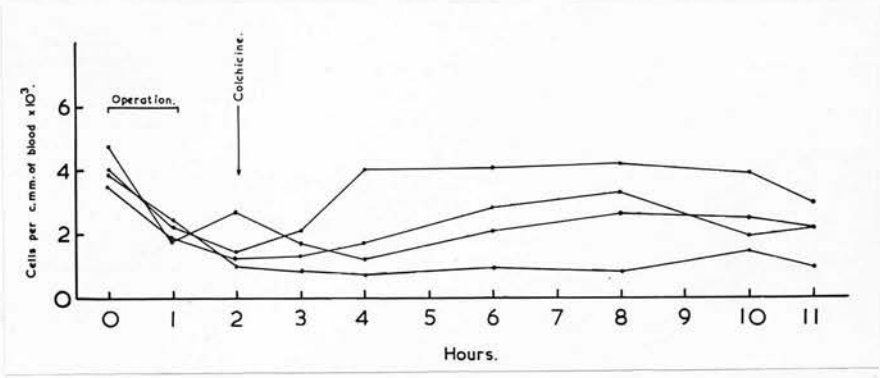

Fig. 28. The effect of colchicine on the number of lymphocytes per cmm. of blood in 4 sham-operated rabbits.

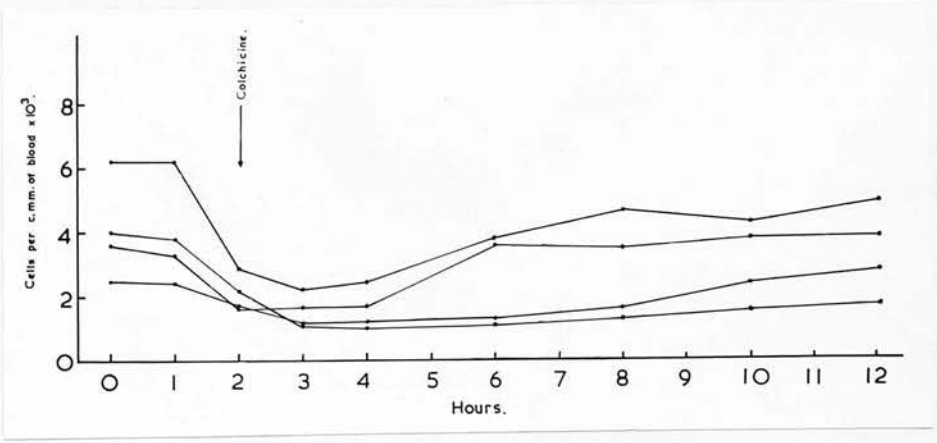

Fig. 29. The effect of colchicine on the number of lymphocytes per cmm. of blood in 4 anaesthetised rabbits.

Table VIII

Table VIII. shows the number of lymphocytes per cmm of blood, and the absolute number of small and large lymphocytes in 4 sham-operated rabbits before and after injection of colchicine.

Rabbit No.	Variables	Hours								
				Colchicine ↓						
		0	1	2	3	4	6	8	10	12
SC1	Total lymphocytes/cmm.	3,505	1,953	1,260	1,358	1,769	2,833	3,296	1,980	2,100
	Small lymphocytes/cmm.	2,945	1,638	1,080	1,221	1,616	2,560	3,040	1,760	1,750
	Large lymphocytes/cmm.	561	315	180	137	153	273	256	220	350
SC2	Total lymphocytes/cmm.	4.004	2,245	1,495	2,132	4,040	4,185	4,266	3,976	2,943
	Small lymphocytes/cmm.	3,646	2,009	1,269	2,054	3,856	3,780	3,870	3,743	2,675
	Large lymphocytes/cmm.	358	246	226	78	184	405	396	233	268
SC3	Total lymphocytes/cmm.	3,905	2,496	1,028	884	777	953	800	1,485	949
	Small lymphocytes/cmm.	3,321	2,016	835	792	609	812	720	1,353	748
	Large lymphocytes/cmm.	584	480	193	92	168	140	80	132	201
SC4	Total lymphocytes/cmm.	4,794	1,855	2,720	1,749	1,297	2,015	3,646	3,350	3,100
	Small lymphocytes/cmm.	3,740	1,365	2,080	1,378	1,004	1,690	3,238	3,047	2,281
	Large lymphocytes/cmm.	1,054	490	640	371	293	325	408	403	819

Table IX

Table IX. shows the number of lymphocytes per cmm. of blood, and the absolute number of small and large lymphocytes in 4 anaesthetised rabbits before and after colchicine injection.

Rabbit No.	Variables	Hours 0	1	2 ↓ Colchicine	3	4	6	8	10	12
AC1	Total lymphocytes/cmm.	3,623	3,350	1,645	1,711	1,770	3,615	3,584	3,840	3,980
	Small lymphocytes/cmm.	3,243	3,000	1,400	1,593	1,635	3,331	3,424	3,690	3,800
	Large lymphocytes/cmm.	380	350	245	118	135	284	160	150	180
AC2	Total lymphocytes/cmm.	6,221	6,280	2,976	2,291	2,459	3,823	4,725	4,352	5,125
	Small lymphocytes/cmm.	5,746	5,800	2,528	2,175	2,211	3,575	4,575	4,148	4,850
	Large lymphocytes/cmm.	475	480	448	116	248	248	150	204	275
AC3	Total lymphocytes/cmm.	4,088	3,820	2,254	1,111	1,060	1,138	1,378	1,600	1,620
	Small Lymphocytes/cmm.	3,341	3,200	2,126	997	980	1,015	1,251	1,344	1,350
	Large lymphocytes/cmm.	657	620	128	114	80	123	127	256	275
AC4	Total lymphocytes/cmm.	2,565	2,400	1,691	1,188	1,224	1,335	1,676	2,499	2,850
	Small lymphocytes/cmm.	2,160	2,000	1,431	1,026	999	1,212	1,480	2,289	2,605
	Large lymphocytes/cmm.	405	400	260	162	225	123	196	210	245

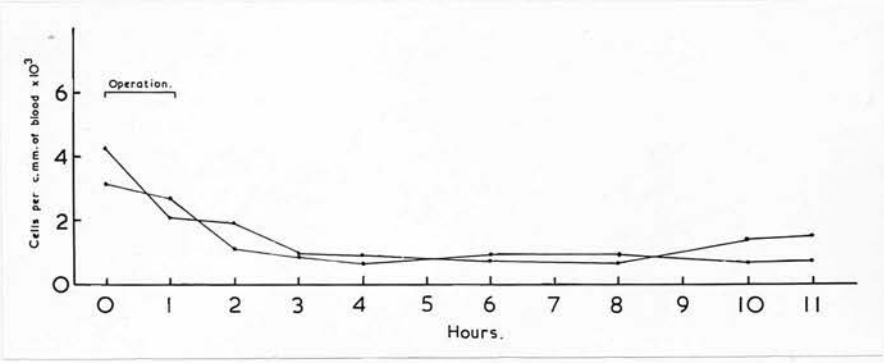

Fig. 30. The effect of sham-operation alone (without colchicine) on the number of lymphocytes per cmm. of blood in 2 rabbits.

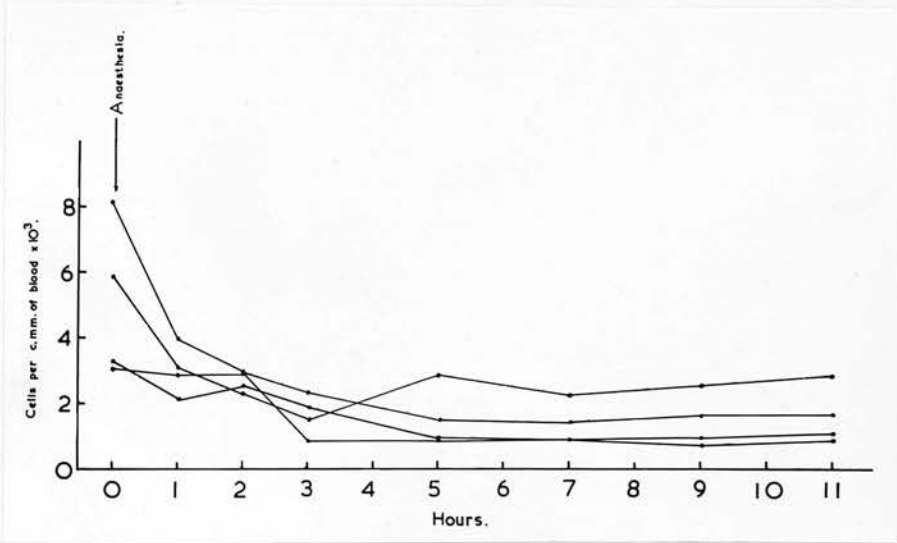

Fig. 31. The effect of anaesthesia on the number of lymphocytes per cmm. of blood in 4 rabbits.

Table X.

Table X. shows the number of lymphocytes per cmm. blood, and the absolute number of small and large lymphocytes in 2 sham-operated rabbits.

Rabbit No.	Variables	Hours								
		0	1	2	3	4	6	8	10	11
01	Total lymphocytes/cmm.	4,247	2,030	1,989	924	978	780	650	1,415	1,500
	Small lymphocytes/cmm.	3,937	1,816	1,747	770	863	660	585	1,273	1,350
	Large lymphocytes/cmm.	310	214	242	154	115	120	65	142	150
02	Total lymphocytes/cmm.	3,146	2,703	1,170	855	614	935	964	646	770
	Small lymphocytes/cmm.	2,717	2,358	1,050	713	502	735	772	470	590
	Large lymphocytes/cmm.	429	345	120	142	112	200	192	176	180

Table XIa.

Table XIa. shows the number of lymphocytes per cmm. blood, and the absolute number of small and large lymphocytes in 4 anaesthetised rabbits, without colchicine.

Rabbit No.	Variables	Hours							
		0	1	2	3	5	7	9	11
A1	Total lymphocytes/cmm.	3,016	2,844	2,950	2,393	1,513	1,484	1,627	1,650
	Small lymphocytes/cmm.	2,365	2,172	2,095	1,668	1,089	o,035	1,083	1,100
	Large lymphocytes/cmm.	651	672	855	725	424	449	544	550
A2	Total lymphocytes/cmm.	3,234	2,138	2,574	1,800	960	880	720	895
	Small lymphocytes/cmm.	2,838	1,733	2,281	1,575	780	770	600	750
	Large lymphocytes/cmm.	396	405	293	225	180	110	120	145
A3	Total lymphocytes/cmm.	8,166	3,920	2,870	861	846	824	910	1,020
	Small lymphocytes/cmm.	7,461	3,500	2,467	738	705	618	700	795
	Large lymphocytes/cmm.	705	420	403	123	141	206	210	225
A4	Total lymphocytes/cmm.	5,880	3,040	2,250	1,488	2,976	2,271	2,530	2,815
	Small lymphocytes/cmm.	4,920	2,506	2,000	1,288	2,539	1,765	1,725	2,005
	Large lymphocytes/cmm.	960	534	250	200	437	506	805	810

pronounced lymphopaenia lasting for the entire experimental period.

It appears therefore that colchicine will depress for a short time the number of lymphocytes per cmm. blood if the initial level is high: when the initial level of lymphocytes is low, either naturally or due to the operative interference, then there is no alteration in this level after the injection of colchicine. Anaesthesia, with or without operation, depresses the level of the lymphocytes in blood.

b) The relative numbers of small and large lymphocytes. It may be seen from Tables VII-XI that the lymphopaenia which occurs in each of the experimental groups is caused by a reduction in numbers of both the small as well as of the large lymphocytes.

c) Morphological characters of the lymphocytes. In the experimental groups in which colchicine was administered, about from 95 to 97% of the lymphocytes seen in blood smears were normal in character and about from 3 to 5% cells showed nuclear abnormalities similar to the ones seen in the peripheral blood described in Part I. Only on one occasion was a large lymphocyte seen showing a mitotic figure. In the thoracic duct lymph as many as 2.5% of the large lymphocytes showed mitotic figures. It was thought that probably these large cells with mitotic figures were either caught up in the lungs before entering the systemic circulation or might have accumulated in the liver or spleen.

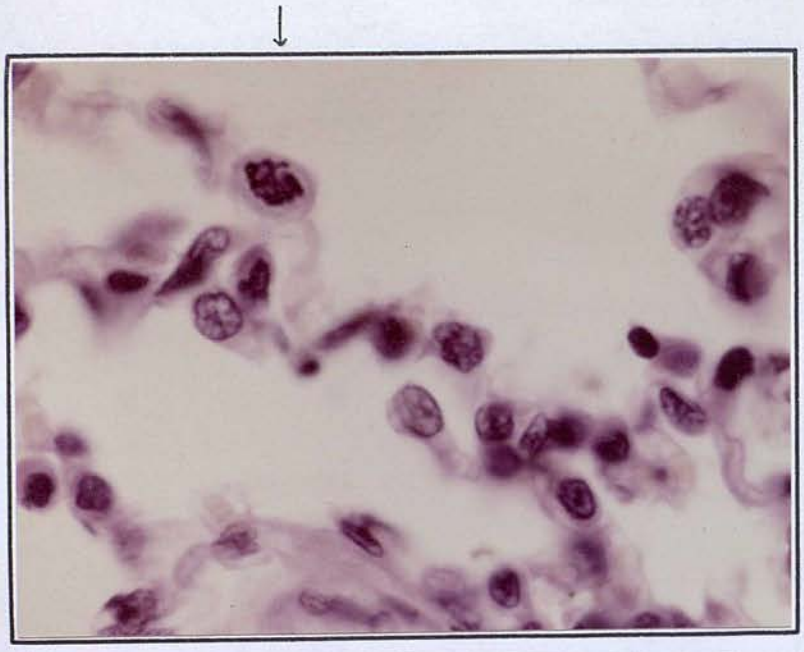

Fig. 32. Section of lung from a rabbit taken 10 hours after the injection of colchicine and 11 hours after lymph drainage. Mitotic figures may be seen in some of the large cells. Susa fixation. Haemotoxylin and eosin x 1000.

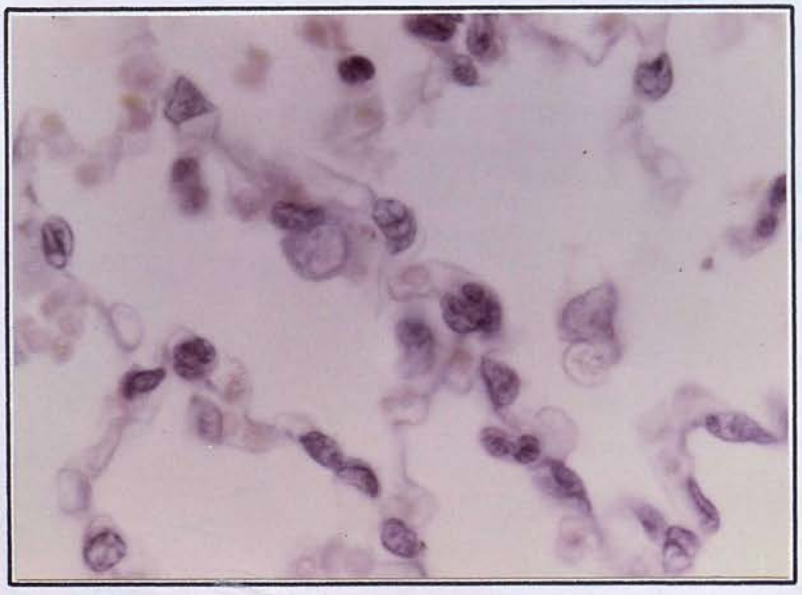

Fig. 33. Section of lung from a rabbit taken 11 hours after lymph drainage alone. No mitotic figures can be seen. Susa fixation. Haematoxylin and eosin x 1000.

3. EXAMINATION OF LUNGS

Examination of the sections of lungs from animals treated with colchicine revealed a few cells with mitotic figures lying amongst the alveolar septa and the surrounding connective tissue (Fig. 32). These cells were not seen in the lungs of animals which were not injected with colchicine (Fig. 33).

It is difficult to decide whether these cells in mitosis are the large lymphocytes which have been removed from the blood of the colchicine-treated animals or whether it is a general effect of colchicine in causing an arrest of mitosis at the metaphase stage of the cells normally present in the lungs.

4. EXAMINATION OF SPLEEN

Similar histological changes were seen as those shown in Fig. 20. No accumulation of large lymphocytes with mitotic figures were seen.

5. EXAMINATION OF LIVER

A search for the large lymphocytes with mitotic figures in sections of the liver yielded negative results.

Part III: The relation of lymphocytes and antibody formation

EXPERIMENTAL METHODS

The methods used in these experiments include those given in Part I. of the present work (Pp. 4-16). In addition the following methods were also used.

1. IMMUNIZATION OF RABBITS

a) The antigen. Formol-killed Salmonella typhi H antigen (Wellcome Research Laboratories) was used in view of the simplicity of assay of anti-H antibody and the large amount of previous work using this antigen. The antigen suspension containing 400×10^6 organisms per ml. was centrifuged at 4000r.p.m. for half an hour. The supernatant was removed. The organisms were then resuspended in a calculated amount of sterile water so as to get a concentration of 10^9 per ml. of the organisms.

b) Immunization procedure. Young adult rabbits of both sexes were used weighing from 2.0 to 3.0kg. Before any experiment, the rabbit to be used was bled from the ear vein and the serum obtained assayed for agglutinins to H antigen of Salmonella typhi. Each rabbit was then given a total of 3×10^9 organisms, 1×10^9 being injected intravenously, intraperitoneally and subcutaneously respectively into the left lower quadrant of the anterior abdominal wall. In

some groups of experiments, subsequent injections of antigen were given to provoke a secondary response as detailed later.

2. COLLECTION OF LYMPH

Lymph was collected from the thoracic duct as in the previous experiments (Part I.) for a period of two hours before the injection of colchicine. Colchicine was then injected in the usual way and hourly samples of lymph were collected for a period of 8 hours. A routine examination of the lymph for volume per hour, total cell counts and differential cell counts was made.

a) Preparation of lymphocyte extract. Lymphocytes were separated from the lymph after a cell count etc. had been made, by centrifuging at 600r.p.m. for 5 minutes. The lymph plasma was then pipetted off as far as was possible. The volume of the cells was determined by using the formula of Harris and Ehrlich (1942) and this was always verified by finding the relative volume of the cells in a haematocrit tube. To the cells, saline was added in an amount which would give a final "dilution" of from 40 to 80. In this way a cell suspension varying from 2 to 4ml. was obtained for homogenizing. This was carried out by the Potter homogenizer using a nylon plunger rotated by an electric motor. The cell suspension was homogenized for 60 seconds. The tube was immersed in ice-cold water during the procedure.

The homogenate was centrifuged and the clear supernatant removed and assayed for antibody. Microscopical examination of the deposit showed few remaining intact cells.

b) Staining of lymph smears. Smears were fixed in methyl alcohol. They were then stained by the usual May-Grunwald Giemsa method.

3. ANTIBODY ASSAY

Antibody estimation on serum, lymph plasma and lymphocyte extract were carried out by the doubling dilution method against a standard agglutinable suspension of Salmonella typhi H*-antigen, equal volumes of each of these and this suspension being incubated in water bath for 2 hours at 56° C. Readings were taken 30 minutes after the tubes had been removed from the water bath.

The different degrees of agglutination were recognised, but not recorded in the results. Any agglutination, from a coarse flocculum at the bottom of the Dreyer tube to a suspension of particles just visible to the naked eye was taken as indicating a positive answer. The highest dilution showing agglutination was considered as the titre. This value was multiplied by 2(as the serum, lymph plasma or cell dilutions in the test tubes are further diluted with an equal volume of the standard agglutinable suspension of antigen), and recorded as the titre.

Blood serum was obtained in the following way. From

* Wellcome Research Laboratories

1.0 to 1.5ml of blood was collected, in a centrifuge tube, from the marginal ear vein of the rabbit. The blood was allowed to clot at room temperature or at 37°C. The clot was freed from the side of the tube, which was then centrifuged. Clear, or occasionally slightly haemolysed, serum was obtained and antibody assays carried out.

4. PREPARATION AND TRANSFER OF THE THORACIC DUCT CELLS

In some experiments, rabbits were immunized with S. typhi (H antigen) and, during the period of the immune response, thoracic duct lymph obtained by operation as described above. From 10.0 to 14.0ml. of thoracic duct lymph was usually collected over a period of from 2½ to 3 hours. Unnecessary bacterial contamination was avoided, the collecting tubes and pipettes being sterile, but no true aseptic methods were used.

The cells were kept alive (i) by using a freshly prepared 1% sterile solution of dry Heparin (Boots), in water, as an anticoagulant. Although the solution of heparin used previously (Liquemin Roche) preserved the cells, it was thought that the phenol present in this solution as a preservative would affect the viability of the cells, (ii) by storing lymph at about 4°C. during the time of collection and (iii) by avoiding air bubbles when pipetting the lymph, for these tend to break up the cells.

From 2.0ml. to 4.0ml. of the lymph so collected was used for the routine examination of the lymph and antibody assay,

and the remaining volume was used for the actual transfer.

It was gently but thoroughly mixed and then divided into two centrifuge tubes. These were centrifuged at 600r.p.m. for 5 minutes to spin down the cells. The supernatant lymph plasma was removed as far as possible. Examination of this revealed a few cells only. To each of the tubes, 1.5ml. of freshly prepared cold Tyrode solution was added gradually, and between each addition the tubes were gently agitated to break up the pellets of cells. The suspension of cells in one of the tubes was injected into one of a pair of recipient rabbits, 0.5ml. intravenously and 1.0ml. intraperitoneally. The suspension of cells in the other tube was heated in a water bath at 56°C. for 30 minutes. It was then injected as above into the other recipient animal, 0.5ml. intravenously and 1.0ml. intraperitoneally. In these experiments, the recipient pairs were always of the same sex and approximate weight.

The time taken from the collection of lymph to the actual transfer was usually from 3 to 3½ hours.

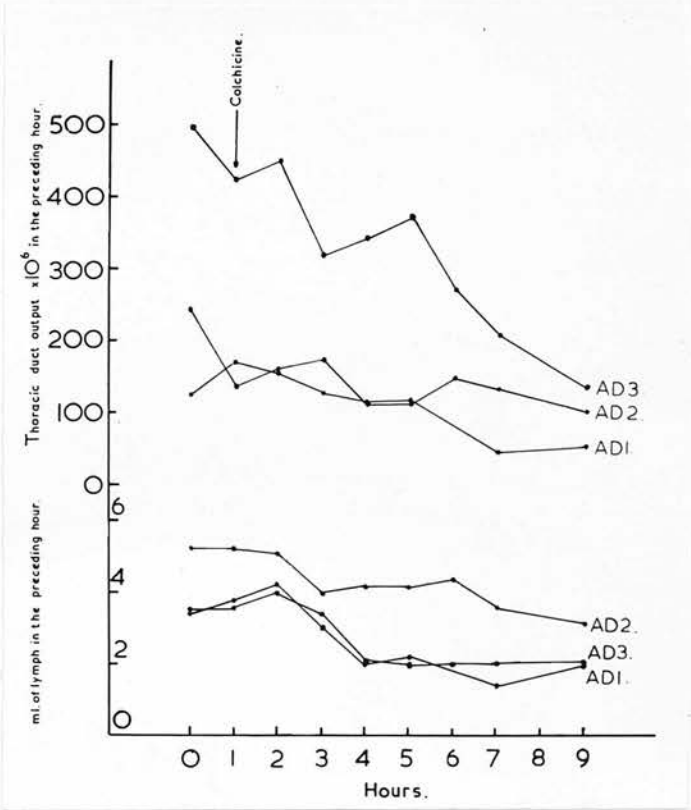

Fig. 34. The effect of colchicine on the rate of thoracic duct lymph flow and the cell output per hour in 3 immunized rabbits.

Part III: The relation of lymphocyte and antibody formation

RESULTS

The results of analysis of the samples of thoracic duct lymph and of peripheral blood obtained over a period of 9 hours in 3 immunized and anaesthetised rabbits are given below and are shown in the accompanying Figs. 34-38 and Tables XII-XVII. Each rabbit was immunized against S. typhi, H-antigen, by injecting 8 days prior to operation, 10^9 organisms intravenously, intraperitoneally and subcutaneously, a total of 3×10^9 organisms. In each of the animals, thoracic duct lymph was collected as decribed above for a period of 2 hours and then 2.5mg./kg. body weight of colchicine was injected intravenously.

1. EFFECT OF COLCHICINE ON THE RATE OF LYMPH FLOW AND THE TOTAL NUMBER OF CELLS COMING DOWN THE THORACIC DUCT PER HOUR IN IMMUNIZED RABBITS

a) Rate of lymph flow. In these three animals, the flow of lymph from 1 to 2 hours after the injection of colchicine decreased over the experimental period of 9 hours.

b) Cells per hour. It can be seen from Fig. 34 and Table XII that the total number of cells per hour coming down the thoracic duct decreases rapidly in No. AD3 and more gradually in Nos. AD1 and AD2 after the injection of the alkaloid.

Table XII.

Table XII. shows the total number of cells and the absolute numbers of small and large lymphocytes coming down the thoracic duct per hour, and the percentage of the large lymphocytes, in the three immunized rabbits, before and after the injection of colchicine.

Rabbit No.	Variables	Hours								
		0	1 (Colchicine ↓)	2	3	4	5	6	7	9
AD1	Total cells x 10^6	123.3	170.2	157.3	124.5	115.2	116.1	-	43.1	55.0
	Small lymphocytes x 10^6	109.7	150.8	140.5	110.2	106.2	106.7	-	38.5	48.5
	Large lymphocytes x 10^6	13.6	19.4	16.8	14.3	9.0	9.4	-	4.6	7.5
	% Large lymphocytes	11.4	11.4	10.9	11.7	8.0	8.0	-	10.6	11.8
AD2	Total cells x 10^6	243.3	136.8	160.0	175.9	114.5	114.5	150.5	136.0	105.0
	Small lymphocytes x 10^6	235.4	128.7	151.0	169.1	108.7	109.2	145.5	132.0	100.8
	Large lymphocytes x 10^6	7.9	8.1	9.0	6.8	5.8	5.3	5.0	4.0	4.2
	% Large lymphocytes	3.2	5.6	5.0	3.7	4.8	4.5	3.1	2.5	4.0
AD3	Total cells x 10^6	497.9	423.8	450.1	319.0	340.2	375.5	268.4	207.9	137.6
	Small lymphocytes x 10^6	468.1	391.4	319.5	294.0	318.1	354.5	248.6	190.8	120.8
	Large lymphocytes x 10^6	29.8	32.4	30.6	25.0	22.1	21.0	19.8	17.1	16.8
	% Large lymphocytes	5.8	7.6	6.6	7.6	6.5	5.6	7.0	8.0	12.0

In the immunized rabbit, colchicine may diminish the rate of lymph flow and decrease the output of the cells from the thoracic duct.

2. EFFECT OF COLCHICINE ON THE TYPES OF CELLS OF THE THORACIC DUCT

a) Relative numbers of small and large lymphocytes. Table XII gives the relative and absolute numbers of the small and large lymphocytes passing down the thoracic duct. It can be seen that the absolute numbers of both small and large lymphocytes decreases gradually after colchicine injection and that the percentage of these cells therefore remains constant throughout the experiment. The percentage of large lymphocytes ranges from 2.5 to 12.0% of the total cells of thoracic duct.

b) Effect of colchicine on the morphology of the thoracic duct lymphocytes in the immunized rabbits. The morphology of the cells of thoracic duct lymph was studied in smears stained with May-Grunwald Giemsa method and in the counting chamber, stained by the brilliant cresyl-blue cyanide method.

Table XIII shows that the majority of cells are the normal lymphocytes. They contribute from 96.6 to 99.5% of the total cells of thoracic duct. These normal cells have the same morphological characters as those of the normal lymphocytes described in Part I (Pp. 20-21). The remainder 0.5 to 3.4% were the abnormal cells described before (Pp. 22-24) which appeared first in the samples taken

Table XIII.

Table XIII. shows the total cells and abnormal cells coming down the thoracic duct per hour and the percentage of abnormal cells and cells showing mitotic figures; in immunized rabbits before and after injection of colchicine.

Rabbit No.	Variables	Hours								
			Colchicine ↓							
		0	1	2	3	4	5	6	7	9
AD1	Total cells x 10^6	123.3	170.2	157.3	124.5	115.2	116.1	-	43.1	55.0
	Abnormal cells x 10^6	-	-	4.2	3.0	2.0	3.9	-	1.1	0.6
	% 'Abnormal' cells	-	-	2.7	2.4	1.8	3.4	-	2.5	1.1
	% Mitotic cells	-	-	1.8	1.2	1.2	2.8	-	0.8	1.1
AD2	Total cells x 10^6	243.3	136.8	160.0	175.9	114.5	114.5	150.5	136.0	105.0
	Abnormal cells x 10^6	-	-	2.0	0.9	1.6	1.6	2.0	1.0	0.5
	% 'Abnormal' cells	-	-	1.3	0.5	1.4	1.4	1.3	0.7	0.5
	% Mitotic cells	-	-	0.6	0.5	0.9	0.9	1.3	0.4	0.5
AD3	Total cells x 10^6	497.9	423.8	450.1	319.0	340.0	375.5	268.4	207.9	137.6
	Abnormal cells x 10^6	-	-	5.1	6.0	3.2	3.2	2.2	1.9	0.8
	% 'Abnormal' cells	-	-	1.1	1.8	0.9	0.8	0.8	0.9	0.6
	% Mitotic cells	-	-	0.3	1.2	0.6	0.5	0.4	0.9	0.6

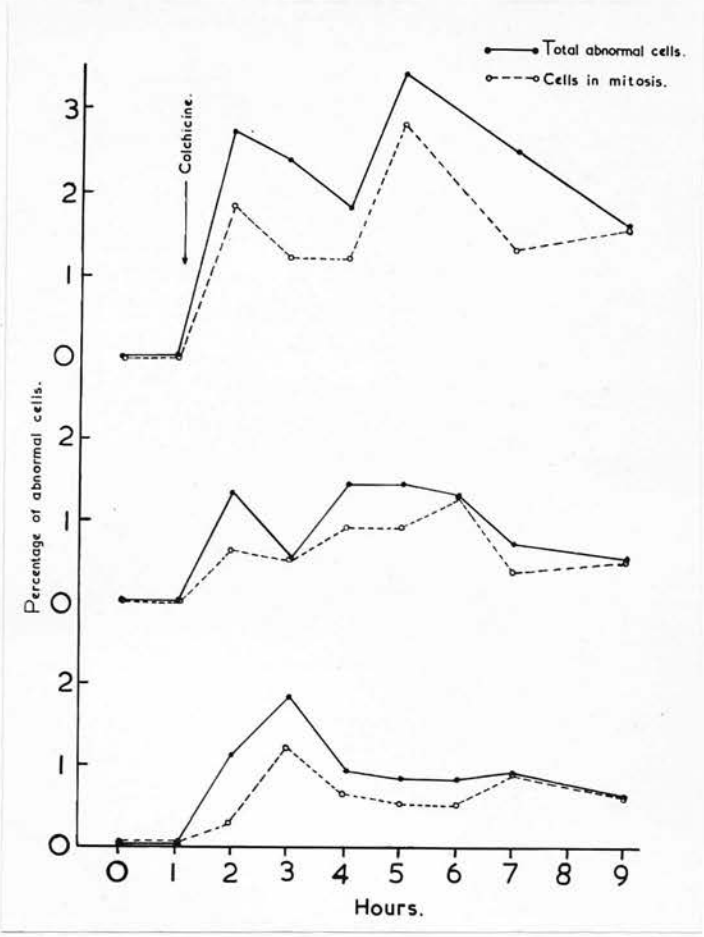

Fig. 35. Percentage of abnormal cells and the cells in mitosis in terms of the total cells of thoracic duct per hour in 3 immunized rabbits.

one hour after the administration of colchicine.

Among the abnormal cells, as may be seen from Table XIII and Fig. 35, the large cells showing mitotic figures at the metaphase stage, predominated and in one animal as many as 2.8% of the total lymphocytes were cells of this type. It may also be noted that from 6 to 7 hours after colchicine injection, the only type of abnormal lymphocytes seen in the thoracic duct lymph were cells in mitosis.

It should also be noted that the lymphocytes coming down the thoracic duct in the immunized rabbits, before the injection of colchicine, showed no obvious differences in morphology when compared with the lymphocytes of the thoracic duct in the normal unimmunized control rabbits. There was no evidence of increased basophilia in the cytoplasm of the cells. The apparently normal lymphocytes and the abnormal lymphocytes seen in the thoracic duct lymph after the administration of colchicine to the immunized animals were similar to the thoracic duct cells seen in unimmunized rabbits receiving colchicine.

3. EFFECT OF COLCHICINE ON THE ANTIBODY LEVEL OF THE BLOOD SERUM, THE THORACIC DUCT LYMPH CELLS AND PLASMA IN 3 IMMUNIZED RABBITS

Table XIV shows the titres of blood serum and lymph cells and plasma. The method of estimating the cell titres is described on p.41-42. It may be seen that the blood serum

Table XIV.

Table XIV. shows the antibody titres of serum, lymph cells and of lymph plasma and the ratio of lymph cells titre to lymph plasma titre, before and after the injection of colchicine in three immunized rabbits.

Rabbit No.	Variables	Hours				
		Colchicine ↓ 0	2	4	6	9
AD1	Serum titre	10,240	20,480	-	20, 480	20, 480
	Lymph cells titre	10,240	10,240	10,240	10,240	10,240
	Lymph plasma titre	2,560	2,560	2,560	2,560	1,280
	Ratio	4	4	4	4	8
AD2	Serum titre	5,120	10,240	-	10,240	10,240
	Lymph cells titre	5,120	5,120	5,120	5,120	2,560
	Lymph plasma titre	1,280	1,280	1,280	1,280	1,280
	Ratio	4	4	4	4	2
AD3	Serum titre	5,120	10,240	-	10,240	10,240
	Lymph cells titre	5.120	10,240	5,120	5,120	5,120
	Lymph plasma titre	2,560	2,560	2,560	2,560	2,560
	Ratio	2	4	2	2	2

and cells appear to have similar titres, differing, if at all, by only one tube. A considerable difference can however be seen between the lymph plasma titres and the serum and cell titres, the lymph plasma titres being always significantly lower than the other two.

The level of the antibodies of serum, thoracic duct cells and plasma remains constant throughout the experimental period. It appears therefore that colchicine has, within the period of observation, no effect on the antibody levels existing in serum, thoracic duct cells and lymph plasma.

4. EFFECT OF COLCHICINE ON THE PERIPHERAL BLOOD OF THE 3 IMMUNIZED RABBITS WITH THORACIC DUCT DRAINAGE

a) The total and differential leucocyte counts. Figs. 36-38 show the response of the total peripheral blood leucocytes and the number of granulocytes and lymphocytes per cubic millimetre of blood following the administration of colchicine in the 3 operated and immunized rabbits. It can be seen that the effects on the blood leucocytes in general are similar to the ones obtained in non-immunized operated animals receiving colchicine as reported in Part I.(Pp. 24-28).

It may be seen from Table XV and XVI that, before the injection of colchicine, the percentage of the large lymphocytes relative to the total number of lymphocytes in blood of these 3 immunized rabbits is 18.1, 23.5 and 23.9 respectively (mean 21.8%). During the preparation of these Tables it

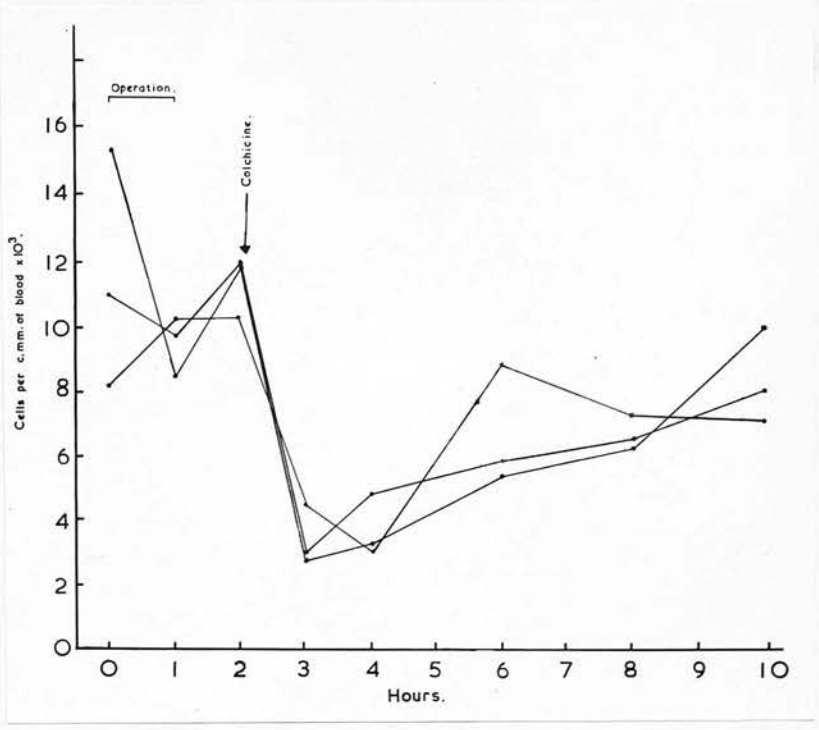

Fig. 36. The effect of colchicine on the total leucocyte counts per cmm. of blood in 3 immunized rabbits in which the thoracic duct was drained.

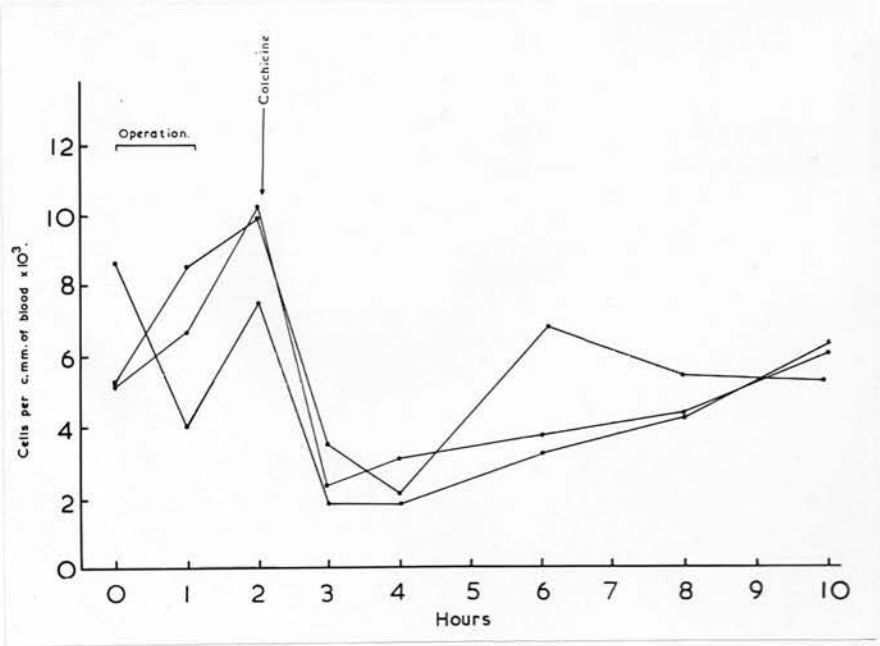

Fig. 37. The effect of colchicine on the number of granulocytes per cmm. of blood in 3 immunized rabbits in which the thoracic duct was drained.

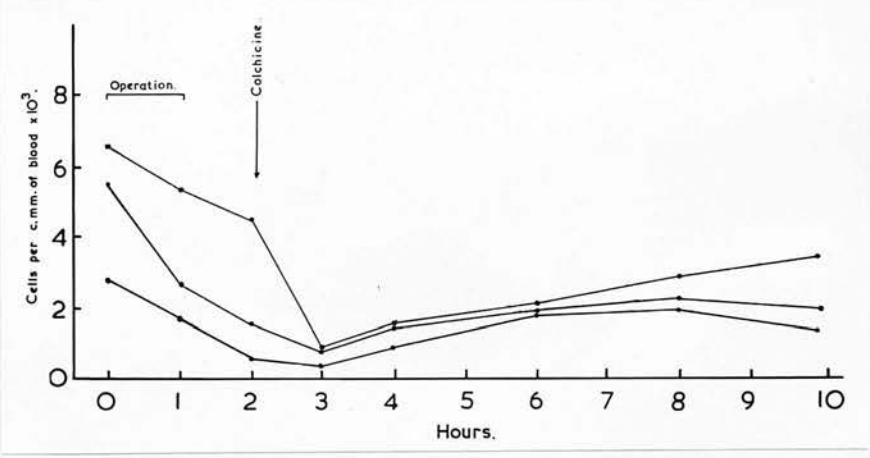

Fig. 38. The effect of colchicine on the number of lymphocytes per cmm. of blood in 3 immunized rabbits in which the thoracic duct was drained.

Table XV.

Table XV. shows the total number of lymphocytes, the absolute numbers of small and large lymphocytes per cmm. blood and the percentage of large lymphocytesbefore the injection of colchicine in three immunized rabbits, and after the injection of colchicine.

Rabbit No.	Variables	Hours							
				← Colchicine ↓					
		0	1	2	3	4	6	8	10
AD1	Total lymphocytes per cmm.	6588	5299	4450	825	1491	2106	1922	3455
	Small lymphocytes per cmm.	5038	4403	3550	711	1289	1836	1702	3188
	Large lymphocytes per cmm.	1550	896	900	114	202	270	220	347
	% Large lymphocytes	23.5	16.9	20.2	12.5	13.5	12.8	11.4	10.4
AD2	Total lymphocytes per cmm.	5445	2632	1520	728	1416	1825	2216	2000
	Small lymphocytes per cmm.	4455	2146	1045	604	1128	1136	1236	1100
	Large lymphocytes per cmm.	990	486	475	124	288	689	980	900
	% Large lymphocytes	18.1	18.4	31.2	17.0	20.3	37.7	42.2	45.0
AD3	Total lymphocytes per cmm.	2747	1742	515	390	840	1891	1922	1370
	Small lymphocytes per cmm.	2092	1332	412	340	720	1493	1668	1130
	Large lymphocytes per cmm.	655	410	103	50	120	398	256	240
	% Large lymphocytes	23.9	23.5	20.0	12.9	14.3	21.0	13.3	17.5

Table XVI

Table XVI shows the number of small and large lymphocytes per cmm. blood and per hour in thoracic duct lymph, also the percentages of large lymphocytes in peripheral blood and thoracic duct lymph, at 0 hours, in 3 immunized rabbits AD1 to AD3 and 10 unimmunized rabbits TD1 to TD6 and C1 to C4.

	Peripheral blood			Thoracic Duct Lymph		
Rabbit	Small lymphocytes 1 cmm.	Large lymphocytes 1 cmm.	Percentage large lymphocytes	Small lymphocytes x 10^6	Large lymphocytes x 10^6	Percentage large lymphocytes
AD1	5038	1550	23.5	109.7	13.6	11.4
AD2	4455	990	18.1	235.4	7.9	8.1
AD3	2092	665	23.9	468.1	29.8	5.8
Mean	3861.6	1068.3	21.8	271.1	17.1	8.4
TD1	5040	450	8.2	327.9	13.7	4.0
TD2	3340	450	11.8	127.5	5.6	4.0
TD3	8530	720	7.8	53.1	1.9	4.0
TD4	5900	264	4.3	69.3	6.4	8.4
TD5	6598	422	6.0	324.9	30.1	8.5
TD6	5955	356	5.6	310.5	42.0	11.4
C1	5148	390	7.1	187.2	8.6	4.9
C2	2784	240	16.3	100.4	9.6	8.0
C3	2162	494	17.6	28.5	5.0	15.4
C4	5885	585	9.1	137.5	10.6	7.2
Mean	5134.2±1917.0	437.1±142.8	9.4±4.5	166.7±115.7	13.4±12.7	7.6±3.6

was noted that the corresponding figures for 10 unimmunized rabbits range between 5.6 and 17.6% with a mean of 9.4±4.5% (Table XVI).

It may also be seen from Table XVI that the absolute number of large lymphocytes in the blood at the start of the experiment are higher in the immunized rabbits than those in the unimmunized rabbits, while the numbers of small lymphocytes are lower. There are no obvious differences in the proportions of absolute numbers of small and large lymphocytes in the thoracic duct lymph.

The number of immunized animals is too small to state that immunization increases the numbers of large lymphocytes and the proportion of large relative to small lymphocytes in the blood: but the possibility should be investigated further.

On examination of the blood smears for a differential leucocyte count, it was noted that about 5.0% of the cells were small lymphocytes exhibiting nuclear abnormalities similar to the ones seen in the unimunized operated animals receiving colchicine (Pp. 25-26). Only on one occasion was a large lymphocyte seen with a mitotic figure.

b) <u>The effect of colchicine on the erythrocytes of the of the peripheral blood</u>. The changes in the circulating erythrocytes correspond to those seen in the previous experiments in unimmunized rabbits (Pp. 28-29).

5. GROSS AND MICROSCOPIC EXAMINATION OF THE SPLEEN IN IMMUNIZED COLCHICINE-TREATED RABBITS

a) Gross findings. The spleen weights in the 3 immunized animals receiving colchicine are shown below. The spleen was weighed 9 hours after the duct was cut.

Rabbit	Spleen weight mg./kg. body weight
AD1	412.2
AD2	448.2
AD3	459.1
	439.8±23.15mg./kg. body weight

It can be seen that the spleen weights are not significantly different from the spleen weights in the 4 normal control animals having had the lymph drained for 11 hours. The spleen weight in those rabbits was 378.1±81.3mg./kg. body weight (Table VI). There is however a significant increase in the weights of spleen of the immunized rabbits receiving colchicine when compared to the ones in the 6 unimmunized rabbits receiving colchicine, in which the weight was 223.2±58.7mg./kg. body weight. (Table VI).

b) Microscopic findings. A few large lymphocytes in mitosis were seen in the imprints. No examination of spleen sections was made.

It was decided to extend these studies on lymphocytes and antibody formation by transferring thoracic duct lymphocytes from immunized donors to normal unimmunized recipients and to follow up the appearance of the specific antibody in the sera of the recipients.

Transfer of lymphocytes from immunized rabbits into normal recipients

Rabbits were immunized with 3×10^9 organisms of S. typhi (H antigen), 10^9 being injected into each of three sites intravenously, intraperitoneally and subcutaneously into the left lower abdomen. Ten days later, the same number of organisms were given in the same manner in order to evoke a secondary response. On the fifth day after the 2nd injection, the thoracic duct lymphocytes were transferred to the normal rabbits.

In a preliminary experiment 2×10^8 of unheated thoracic duct lymphocytes were injected intravenously into one of a pair of recipient rabbits and the same number of heat killed cells into the other recipient animal. No antibodies could be demonstrated in the sera of the two animals over a period of 10 days. This failure of the appearance of antibodies was thought to be due possibly to injury of the cells because of technical mistakes. The remainder of the experiments were more successful.

The results of the transfer of thoracic duct lymphocytes into a group of 15 recipient rabbits are given below and are shown in the accompanying Figs. 39a and 39b. and Table XVII. The antibody titres of the blood serum, cells and lymph plasma of the donor rabbits are also reported in Table XVII. A total of from 1.2 to 2.9 x 10^8 of thoracic duct lymphocytes, having from 1.10 to 2.7 x 10^8 small lymphocytes and approximately from 0.08 to 0.22 x 10^8 large lymphocytes, were injected intravenously and intraperitoneally into each of the recipient rabbits. Out of the 15 rabbits, 7 acted as controls and received thoracic duct lymphocytes which were killed by heating in a water bath at 56°C for 30 minutes, and 8 animals were injected with the living lymphocytes. The serum antibody titres were estimated on 1, 2, 3, 4, 5, 6, 7, 9, 11, 13, 15, 18, 21, and 24 days after the injection. In each of the experiments, except No. A8 the recipient rabbits were in pairs of the same sex and approximately the same weight: one of the pair received killed cells and the other the live cells. The methods used are described on Pp. 43-44.

In experiment No. A8 no corresponding control animal was used because a sufficient amount of lymph could not be collected from the donor rabbit as it died 2 hours after lymph drainage.

Table XVII

Table XVII shows the total number of thoracic duct lymphocytes and the relative numbers of the small and large lymphocytes injected intraperitoneally and intravenously, into each of the 15 recipient rabbits. It also shows the antibody titres of the sera, thoracic duct lymphocytes and lymph plasma of the donor rabbits.

Recipients	Total cells x 10^8	Small lymphocytes x 10^8	Large lymphocytes x 10^8	DONORS' TITRES Blood Serum	Cells	Plasma
A1 and K1	2.03	1.81	0.22	5120	1280	640
A2 and K2	2.90	2.72	0.18	10240	20480	5120
A3 and K3	2.10	1.89	0.21	5120	2560	1280
A4 and K4	2.00	1.75	0.15	81920	81920	20480
A5 and K5	2.50	2.35	0.15	40960	40960	20480
A6 and K6	1.20	1.12	0.08	20480	10240	2560
A7 and K7	1.40	1.30	0.10	20480	10240	2560
A8	2.50	2.30	0.20	10240	5120	1280

A1 to A8 received living thoracic duct lymphocytes
K1 to K8 received heat-killed thoracic duct lymphocytes
A4 and K4)
A5 and K5) received cells from the same donor rabbit
A6 and K6)
A7 and K7) received cells from the same donor rabbit

1. ANTIBODY TITRES OF THE SERUM, CELLS AND LYMPH PLASMA OF DONOR RABBITS

It will be seen from Table XVII that in the donor rabbits the antibody titres in the serum and of the cells are always higher than that of the lymph plasma titres, a finding which is in accord with the result given in the experiments reported above on p. 48.

2. ANTIBODY TITRES OF THE SERA IN THE RECIPIENT RABBITS

Figs. 39a and 39b show the results of antibody estimation in these animals. In one out of the 7 control animals, a pre-existing titre of the antibody specific to H-antigen was present (No. K4). In most of the control animals receiving killed cells, antibody specific to S. typhi, H-antigen, appeared in the sera of the recipient rabbits in very low titres ranging from 2 to 4 on the 1st day after the injection of the thoracic duct lymphocytes. The following day, the titre either fell below the threshold of measurements used in these studies, or the antibody persisted at a low level for from 4 to 9 days. In recipient No. K6, measurable amounts of antibody appeared on the 5th day; none, however, could be demonstrated on the next day. In the recipient No. K4, the response was exceptional; the antibody titre was 16 on the 1st day after injection of the killed lymphocytes and it rose gradually to 64 on the 6th and 7th days;

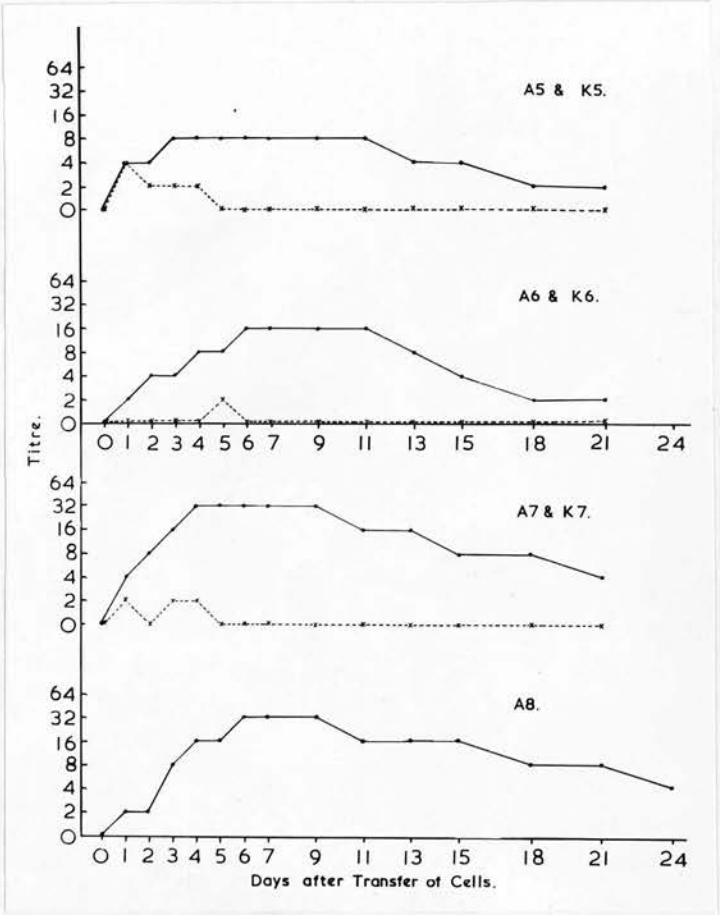

Fig. 39b

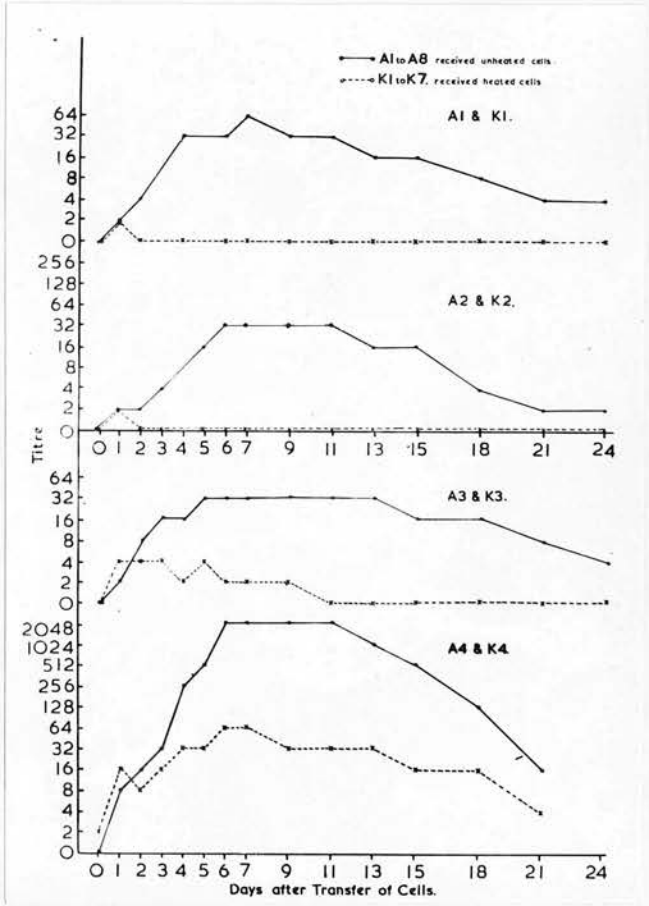

Fig. 39a

Fig. 39a and 39b. Serum antibody titres of 15 rabbits following intravenous and intraperitoneal injection of thoracic duct cells.

thereafter declined, and was not measurable on the 21st day. The antibody titres of the donor rabbit in this experiment were very high.

Antibody was present in the sera of the animals receiving unheated lymphocytes on the 1st day after the transfer. The antibody titres were similar to those of the controls receiving heated cells. Unlike the majority of the controls, however, the serum titres then rose gradually for some days. In 7 of these rabbits, titres of from 8 to 64 were reached by the 6th day. In the recipient rabbit No. A4, (the pair to rabbit No. K4), a peak value of 2048 was attained. By about the 11th day the antibody titres begin to decline and to fall below the threshold of measurement by about the 21st day or even later.

When the pairs of recipient rabbits (K1:A1 - K7:A7) are compared it can be seen that the antibody levels are always considerably higher in the rabbits receiving unheated lymphocytes. The titres in this group rise for some days, maintain this level and then gradually decline from about the 11th day. The titres in the rabbits receiving killed lymphocytes remain low and have usually disappeared by the 9th day or earlier. Animal K4 is an exception; but in this animal also the antibody levels remained significantly (5 or 6 tubes) lower than its pair receiving unheated lymphocytes (A4).

DISCUSSION

The effect of colchicine on the lymphocytes of the thoracic duct and blood discussed in relation to the formation of lymphocytes.

Lymphocytes are produced in the nodes and in other lymphoid tissue scattered over the body. A main site of production is the lymph node as shown by the fact that there are more lymphocytes in the efferent than in the afferent vessels of the lymph glands (Drinker and Yoffey, 1939a). The addition of a large number of lymphocytes to the lymph while passing through a lymph node has been shown by Florey (1926-1927) in the guinea pig, Haynes and Field (1931) in the dog and Baker (1932-1933) in the cat.

Nearly all the lymphocytes which continually escape from the lymph nodes into the lymph enter the circulating blood by way of the thoracic duct and the right cervical and subclavian and the right lymphatic ducts. Blalock, Robinson, Cunningham and Gray (1937), by lymphatic blockage in dogs and cats, showed that the level of the circulating lymphocytes fell almost to zero in experiments where blockage was complete. A few of the lymphocytes may however reach the blood directly by way of the lymphatico-venous communications in the abdomen and thorax (Andreasen and Gottlieb, 1947), or may escape through the stomata in the endothelium of the post-

capillary veins of the lymph nodes (Sanders, Florey and Barnes, 1940).

The total number of cells coming down the lymphatic ducts are sufficient to replace all the lymphocytes of the blood about 11 times a day in the rabbit, giving a life-span of the lymphocytes as about 2 hours only (Hughes, May and Widdicombe, 1956). Since the number of the lymphocytes in the blood remains relatively constant under normal conditions, it follows that over a given period as many leave the circulation as enter it. In spite of the tremendous numbers of cells involved, it is not known for certain where these lymphocytes that leave the peripheral blood are going, although several suggestions have been put forward to explain this. The following brief discussion indicates some of these suggestions.

Wiseman (1931-1932) suggests that the lymphocytes disintegrate shortly after they enter the blood; lymphocytes have however been seen to live for several days in the rabbit's ear chamber (Ebert, Sanders and Florey, 1940).

According to Bunting and Huston (1921), lymphocytes migrate through the intestinal epithelium and leave the organism by way of the lumen of the intestines. Erf (1940) showed that, in rabbits, intravenously injected lymphocytes still disappear in the absense of a gastrointestinal tract. It is difficult to relate experiments on such mutilated animals to the normal physiology of the intact animal.

Andrew and Andrew (1949) believe that the lymphocytes migrate to the epidermis and are transformed there into epithelial cells.

Yoffey and Parnell (1944) consider that the lymphocytes from the blood stream are filtered in the bone marrow and act as the precusors of the other blood cells. Bloom (1928) and others have said that lymphocytes may be transformed into many other types of cell, but Ebert et al. (1940) failed to see such a transformation in the rabbits's ear chamber, while Hall and Furth (1938) and J. Medawar (1940) could see no evidence of transformation in lymphocyte survival experiments in vitro.

Sjövall in 1936 suggested that lymphocytes pass from the blood back into the lymphatic system. This idea, that there may be a recirculation of the lymphocytes, has been supported by some recent work involving labelling of the cells in various ways. Farr (1951) gave autogenous transfusions of lymphocytes labelled with a fluorescent dye. He found that the majority of the cells injected could be seen in the lymphatic tissue 12 hours later. Whether or not these were living lymphocytes could not be decided from his experiments. Ottesen (1954) labelled white cells with P^{32} and showed that the lymphocytes formed two groups: a few cells had a life-span of about 3 days, while the majority of the lymphocytes had a mean age of from 100 to 200 days. It may be that the long life of the majority is explained by a recirculation from the blood back into the lymphatic tissue. Moreover Gowans (1957) has shown that the fall in the

lymphocyte output from the thoracic duct which occurs two days after continuous lymph drainage in the rat can be prevented if living lymphocytes, collected from the duct are returned to the blood. Dead cells had no such effect. The entry of the living lymphocytes into the blood appears to be essential for maintaining the output of the lymphocytes from the thoracic duct: a situation most easily explained by a recirculation of the transfused cells.

Assuming that a recirculation of lymphocytes occurs, the majority of the cells in the thoracic duct lymph would be old lymphocytes rather than the newly formed cells. In this case a mitotic-inhibitor should not reduce appreciably the output of lymphocytes into the blood. If however, recirculation does not occur or occurs on a limited scale, a mitotic-inhibitor should reduce the numbers of lymphocytes entering the lymph <u>en route</u> for the blood, since the lymphocytes will be newly-formed cells. It is possible however that lymphocytes after formation wait in the lymph nodes before they are discharged into the lymph. In this case, a mitotic-inhibitor might not affect the output of cells into the lymph ducts immediately.

In the present experiments the effect of colchicine, a mitotic-inhibitor, on the thoracic duct lymph and its

content of cells was studied. Changes in the peripheral blood, the lungs, spleen and liver were also observed at the same time.

Colchicine was found to have no consistent effect on the rate of lymph flow, but increased the hourly output of the thoracic duct lymphocytes independently of the rate of flow. As much as 50% (or more) increase in the output of cells was observed in rabbits, 2 to 3 hours after colchicine, declining thereafter to reach a level usually slightly below the basal figure.

The increase in the hourly output of both small and large lymphocytes in the thoracic duct lymph after the injection of colchicine may be due to some pharmacological action of the alkaloid other than its anti-mitotic action. One possible mechanism by which colchicine may act is by enhancing gastrointestinal activity by neurogenic stimulation (Ferguson, 1952; Goodman and Gilman, 1955). This may be responsible for the increased cell output into the lymph; splanchnic nerve stimulation is known to increase output of thoracic duct lymphocytes (Florey, 1927).

It is plain however that colchicine caused no significant decrease in the numbers of the thoracic duct lymphocytes throughout the experimental period of 10 hours. If one is not being misled by some over-riding pharmacological action of colchicine other than its anti-mitotic effect,

these experiments show that at least the majority of the lymphocytes of the thoracic duct have not been formed in the preceding few hours. Gowans (1957), from his experiments, argues that the output of the thoracic lymphocytes could be maintained when living but not dead lymphocytes were transfused in cannulated rats for one of three reasons. First the transfused lymphocytes themselves recirculate; secondly the cell-breakdown products stimulated mitosis in the lymph nodes; and thirdly that some of the cells (presumably the large lymphocytes) divided to produce a large number of new lymphocytes. It is unlikely, if our present experiments are applicable to the cannulated rat as well as the rabbits, that either of the last two reasons given are valid, for a mitotic-inhibitor causes no fall in the number of lymphocytes even over a period of 10 hours.

The most striking effect of colchicine on these cells is the appearance of nuclear abnormalities. From 95 to 99% of the lymphocytes looked apparently normal and exhibited no nuclear changes, but from 1 to 5% of the lymphocytes showed obvious nuclear abnormalities. These abnormal cells were seen within an hour or even earlier (20 minutes) in the thoracic duct lymph on the administration of colchicine.

Up to 2.5% of the total cells in the thoracic duct lymph were large lymphocytes showing mitotic figures at various stages of metaphase.

It is interesting to note that mitotic figures were always seen in the large lymphocytes and never in the small lymphocytes of the thoracic duct lymph. This suggests that the large lymphocytes can divide but not the small lymphocytes. This is in agreement with the results of previous workers. Hall and Furth (1938), on the basis of *in vitro* studies on the thoracic duct lymph in rabbits and dogs suggested that the medium and large lymphocytes divided to produce small lymphocytes. Mann and Higgins' (1954) work on the rat's lymph also suggested that a rapidly-dividing large lymphocyte could be responsible for maintaining lymphocyte production in long-term thoracic duct cannulated animals. Ottesen (1954), by using P^{32} for labelling the desoxyribose nucleic acid (DNA) of the cell nuclei, has demonstrated that one type of lymphocyte has a very short life-span in the blood, while the majority of the cells (78 to 89%) have a long life-span of 100 to 200 days. This is consistent with the large lymphocyte being a cell that divides while the small lymphocyte remains undivided for a very long time. Gowans (1957) has shown that the rat's thoracic duct lymph contained about 5% of medium and large lymphocytes which were seen to divide *in vitro*.

Moreover, in more recent work, he has found that tritium-labelled thymidine is incorporated rapidly into the DNA of medium and large lymphocytes but not into the small lymphocyte. (Gowans 1958). Such incorporation would occur during cell division and its absence in the small lymphocyte in the early stages of the experiment shows that this cell has not divided or been formed in that period.

Other abnormalities, particularily those described as karyolysis and pyknosis, were seen in the nuclei of both large and small lymphocytes. Anuclear cell fragments were also found, usually 6μ or less in diameter. Some of these nuclear abnormalities correspond to those reported by Brues and Jackson (1937) and by Roberts, Florey and Joklik (1952) in the liver cells of animals given colchicine after partial hepatectomy. They are presumably due to degenerative changes in the nuclei either before cell-division or during division. In the latter case chromosomal material is scattered in the cytoplasm. There are also some cytoplasmic changes in the large cells: the basophilic material, as seen in stained smears, is collected into a meshwork; the interstices of this mesh are eosinophil. There is also, in many cells, considerable vacuolation. These cytoplasmic changes are also seen in the lymphocytes of peripheral blood. They were also observed in the regenerating livers of mice partially hepatectomized and given colchicine (Roberts, 1958).

The injection of colchicine caused, within an hour, a lymphopaenia when the preinjection level of blood lymphocytes was high. This occurred in unanaesthetised rabbits; it was also seen in the anaesthetised and operated animals, but in these the position is complicated, for anaesthesia alone or anaesthesia and operation cause a marked lymphopaenia by themselves. These results are in agreement with the work of Sanders, Florey and Barnes (1940) and others who observed that any prolonged operation gives rise to a marked lymphopaenia, irrespective of whether the main ducts are cannulated or not. Yoffey (1933), showed that a profound lymphopaenia occurred in anaesthetised animals left quietly for 2 hours.

The fall in blood lymphocytes occurring after colchicine is probably not a specific effect due to the anti-mitotic activity of the alkaloid; the injection of colchicine is possibly one of a large group of stimuli causing lympho-paenia.

The blood lymphopaenia after colchicine administration affects the numbers of both small and large lymphocytes. The majority of the lymphocytes are apparently normal cells but about 5% exhibited nuclear abnormalities of similar nature to those of the thoracic duct lymphocytes. Only an occasional large lymphocyte showing a mitotic figure was seen in the peripheral blood in colchicine-treated animals; it should be noted however that 2.5% of the thoracic duct lymphocytes

draining into the blood at this time are in arrested mitosis. Some explanation as to where these cells go is required. It is possible that they fragment in the blood; this fragmentation may account for the many nuclear masses and smudged cells observed in the blood smears. If this was so, one would expect to find a decrease in the proportion of the circulating large lymphocytes. The blood of some colchicine-treated animals did show such an effect - in others there was no regular change in this direction. It is more probable that the large cells in mitosis were filtered out in the pulmonary circulation. In sections of the lungs, a few large cells in mitosis could be seen. Some of these may have been large lymphocytes. The role of the lungs in removing and sequestrating foreign injected leucocytes and lymphocytes has been demonstrated by various workers (Osogoe, 1950; Weisburger, Guyton, Heinle and Storaasli, 1951; Ambrus, Ambrus, Johnson, Packman, Chernick, Back and Harrison, 1954; Bierman, Kelly and Cordes, 1955). The absence of the many colchicine-mitotic figures in the large lymphocytes of the spleen lends support to the view that the large cells in mitosis seen in the lungs are the large lymphocytes removed from the blood by the lung capillaries before reaching the systemic circulation.

A crucial experiment would be to examine samples of blood removed from the pulmonary artery, the pulmonary veins and from

the cavities of the heart in colchicine-treated animals so as to track the path of large lymphocytes in mitosis coming from the thoracic duct. Preliminary experiments on these lines show that colchicine-mitotic figures can be seen in the blood obtained from the innominate vein but not in systemic arterial blood.

The effect of colchicine on peripheral blood granulocytes with particular reference to the effect of colchicine and anaesthesia

Previous workers have described changes in the level of granulocytes in the peripheral blood after colchicine administration. Accordingly, in the present series of experiments, changes in the blood granulocytes were followed. In the unanaesthetised rabbits, the effect of colchicine was to produce an initial granulocytopaenia, occurring at about 1 hour after the administration of the alkaloid; this granulocytopaenia was followed in the next few hours in the majority of experiments by granulocytosis. These results are in agreement with those of various workers (Dixon and Malden, 1908; Fagraeus and Gormsen, 1953; Harm, 1953). In the anaesthetised rabbits, with or without operative interference, the initial granulocytopaenia produced by colchicine tends to persist without any evidence of a granulocytosis; this has apparently not been noted previously in the literature.

Dixon (1912) thought that the granulocytopaenia was caused by the granulocytes being expelled from the circulation and collecting in the various tissues of the body, especially in the bone marrow and the lungs. No quantitative studies on these two tissues were, however, made. Fagraeus and Gormsen (1953) suggested that the initial granulocytopaenia is probably due to a toxic effect of colchicine causing a peripheral lysis of the granulocytes. Although there were a few ruptured granulocytes in the blood smears of colchicine-treated animals in our experiments, their number did not appear to account for the profound granulocytopaenia which occurs. There was no evidence from sections of the lungs that granulocytes collected in any great numbers in the pulmonary circulation. The cause of the granulocytopaenia following the injection of colchicine remains obscure.

The granulocytosis which follows the initial granulocytopaenia in unanaesthetised animals is considered to be due to the granulocytes re-entering the circulation; at this time the numbers of these cells in the bone-marrow are found to diminish (Dixon, 1912). Fagraeus and Gormsen (1953) suggest that the granulocytosis is due to the irritation which is produced by the toxic effect of colchicine on the blood-producing tissues.

The absence of the granulocytosis in the anaesthetised rabbits could be due to the anaesthetic causing an increased

toxicity of the alkaloid. As there were no other manifestations of increased toxicity in the animals, this seems to be a very unlikely explanation for the persisting granulocytopaenia.

It is possible that changes in the mechanism by which the release of the granulocytes occurs from the leucocyte-forming tissues may be responsible for this granulocytopaenia.

Another way in which colchicine and anaesthesia may be acting is to depress the production of the granulocytes by arresting mitosis. This effect of colchicine to arrest mitosis may be facilitated in the presence of the anaesthesia.

Further studies on this problem are obviously indicated; the normal mechanism of the response of the granulocytes to colchicine - a decrease followed by an increase - must be elucidated first before the alteration of the response in anaesthetised animals can be understood. It would seem to be profitable to examine the blood-forming tissue further in colchicine-treated animals.

In some of the colchicine-treated rabbits the lobulated nuclei of the granulocytes were converted into globular nuclei. This change has been described by Harm (1953) in the heterozygous Pelger rabbit. She showed that the nuclei of the granulocytes of these animals were changed by colchicine from a lobulated into a globular form so that they came to resemble the nuclei of granulocytes from a homozygous Pelger rabbit.

The relation of lymphocytes and antibody formation

The lymphoid tissue of the body has been implicated as the seat of antibody formation. In 1935, McMaster and Hudack showed that antibody may be formed in the lymph nodes. These workers injected one antigen into the right ear of a mouse and a different, but related, antigen into the left ear. They found that specific antibody appeared first and to a higher titre in the lymph node draining the ear injected with the corresponding antigen. Ehrich and Harris (1942) extended these studies further and examined the lymph in the efferent and the afferent vessels of the draining popliteal node of a rabbit into the foot pad of which typhoid antigen or sheep erythrocytes had been injected. They found the appearance of antibody from 2 to 4 days, the titre of antibody reaching its peak after the 6th day. The titre was always higher, by as much as 100 times, in the lymph of the efferent vessel than in the afferent vessel. They found at the same time an increased output of the lymphocytes in the efferent vessels.

Harris, Grimm, Mertens and Ehrich (1945) collected the lymph from the efferent vessel of the popliteal lymph node of a rabbit injected into the foot pad with typhoid antigen or sheep erythrocytes. On analysis of the antibody titres of the cells (99% of the cells were considered by them as lymphocytes) and of the lymph plasma they discovered that the

titre of antibody of the cells was always higher than that of the lymph plasma being in the proportion of 8:1 to 16:1. Further, they thought that the lymphocytes could neither absorb nor adsorb antibody when incubated with plasma containing large amounts, thus showing that the lymphocytes had not passively taken up antibody from the surrounding plasma in their experiments.

Dougherty, Chase and White (1944), White and Dougherty (1945) and Kass (1945) reported that, in mice immunized with sheep erythrocytes, the extracts obtained from the lymph glands yielded large amounts of antibody; the antibody titre was about 6 to 8 times that of the corresponding blood serum. The methods of estimating cell titres have, however, been questioned (Haleb, Endicott, Bell and Spear, 1949).

The lymphoid tissue undoubtedly takes a part in the formation of antibody, but which cell is responsible is more doubtful. The general literature on this subject has been reviewed by Roberts and Gowans (1958). Here the work that has been done on this subject using the technique of cell transfer will be considered and discussed in relation to the present studies on thoracic duct lymphocyte transfers from antibody-producing rabbits.

The general scheme into which these studies fit is that cell suspensions of an immunized donor animal are transferred into normal recipients usually of the same species; the

appearance of antibody in the blood serum is then followed. Such a response in which antibody formation is induced by living transferred cells is called 'adoptive immunity' (Billingham, Brent and Medawar, 1954). The first experiments of this type were conducted by Deutsch (1899). Topley's work (1930) is perhaps the most careful of these earlier studies: he used rabbits and transferred spleen cells, differentiating between passive, active and adoptive immunization.

Chase (1951) showed that when the cells from the lymph nodes of a guinea pig immunized with sheep erythrocytes were injected into a normal recipient, antibody could be demonstrated in the serum of the recipient within a day of transfer of the cells.

In a large series of experiments, the Harris' and their co-workers, (Harris and Harris, 1949; 1950; 1954; Harris, Harris, Beale and Smith, 1954; Harris and Farber, 1954; Harris and Harris, 1955) have transferred suspensions of lymph node cells from the popliteal node of a donor immunized against dysentery bacilli into normal recipient rabbits, the injection being made into the foot pad. Such a transfer results in the appearance of agglutinins in the serum of the recipient. The agglutinin titre rises to reach its peak values on the 3rd day, starts to decline gradually by the 5th day; it is not measurable later than the 23rd to 40th day. Killed or injured cells when transferred failed

to produce this effect.

Other studies by Billingham, Brent and Medawar (1954) and by Mitchison (1955), have shown that an adoptive immune reaction can be produced when living cells are transferred. Mice were used in these experiments and lymph node cells were transferred; the immune reaction studied was that resulting from tissue transplantation. Mitchison (1957) continued these experiments, using spleen cells and a bacterial antigen; he showed that, if the homograft reaction to the transferred cells was circumvented, either by irradiating the recipient mice or by using inbred strains of mice, then the antibody response in the recipient was prolonged beyond 25 days. The transferred cells had obviously lived and continued to produce (or to give rise to other cells which produced) antibody.

The results of these transfer experiments do not indicate which of the lymph node or spleen cells actually form the antibody. A very mixed population of the cells is transferred in these cases. The use of a pure population of one type of cells would obviously clarify the mechanisms involved.

Weeslén (1952) used thoracic duct lymphocytes as a relatively pure population, in studies on antibody formation. He injected into normal recipient guinea pigs a suspension of the lymphocytes separated from the thoracic duct lymph of donor rabbits immunized against horse serum. When an intravenous injection of 0.3ml. of horse serum was made, 4 days after transfer of cells, the guinea pigs showed severe

anaphylactic reactions.

Holub (1958) in similar studies used thoracic duct lymphocytes which were mixed with bacterial antigens, Brucella suis and S. paratyphi B; in the proportion of 2 to 6 organisms per cell. The cells were transferred to newborn rabbits of 2 to 5 days old. These have been shown to be incapable of reacting to antigen by an active immune response.* Antibody could be demonstrated 2 days after the cell transfer and attained a peak titre of 32 or 64 after 5 to 8 days. It would be helpful in the interpretation of these results if independent evidence could be established that rabbits up to 13 days old cannot produce antibody even to such low titres.

In the present work the problem of the role of lymphocyte in antibody formation was approached in two ways. In one, colchicine was used and its effect on the thoracic duct lymphocytes was studied in the immunized rabbits. In the other, thoracic duct lymphocytes from antibody-producing rabbits were transplanted to normal rabbits.

The effects of colchicine were observed in relation to changes in thoracic duct lymph, peripheral blood and spleen and the relative antibody titres of blood serum, lymph plasma and lymph cells.

The rate of flow and the output of cells per hour of

* (Sterzl, 1957)

the thoracic duct lymph were moderately decreased in the immunized rabbits when colchicine was given; both small and large lymphocytes being affected to the same extent. There was no evidence whatsoever of the early peak increase in the cell output seen in the 5 out of 6 of the unimmunized rabbits given colchicine. The mechanism of this altered response is not under-stood. It cannot be said, from such a small series, to what extent the longer term response to colchicine in these immunized rabbits differs from the longer term response seen in normal rabbits: there is no great difference.

The numbers and proportion of large cells coming down the thoracic duct in the immunized rabbits before colchicine administration was about 17 x 10^6 per hour and 8%. These do not differ significantly from the corresponding figures for unimmunized rabbits (13.4±12.7 x 10^6 per hour and 7.6±3.6%). It will be remembered that, in the unimmunized rabbits, large lymphocytes in mitosis formed up to 2.5% of the total cells in the thoracic duct after the injection of colchicine. In the immunized rabbits the proportion was at the most 2.8%; there was thus no significant difference in the number of mitotic cells in the immunized and unimmunized animals. There were no obvious differences between immunized and unimmunized rabbits in the proportion of the small and the large lymphocytes in thoracic duct lymph after colchicine administration. No change in the morphology of the lymphocytes was observed in the immunized rabbits when compared to unimmunized rabbits.

The small number of large lymphocytes in mitosis coupled with no definite increase in the percentage of large lymphocytes in the thoracic duct lymph of immunized rabbits would go against the suggestion of Yoffey and Courtice (1956) that these cells are the 'most likely cells to possess antibody-forming properties'. It was however found, on tabulating the blood counts in the two groups, that the absolute numbers of large lymphocytes in the blood of immunized rabbits were higher than those in the unimmunized rabbits, while the number of small lymphocytes was low. Further investigations are required to establish definitely that there are significantly more large lymphocytes in the blood of rabbits immunized in this way.

The spleen weights in our series of immunized rabbits given colchicine were higher than those of normal rabbits and were much higher than those of unimmunized rabbits given colchicine. The figures, are respectively, 439±23, 378±81 and 223±59mg. per kg. body weight. During immunization the spleen weight increases (Fagraeus, 1948), so our results do not necessarily contradict those of Fagraeus and Gormsen (1953), who found a 50% reduction in the weight of the spleen when colchicine was given to immunized mice.

The estimation of cell antibody titres by homogenization of small volumes of cells and subsequent doubling dilution of the spun supernatant, is open to criticism (Haleb et al., 1949). However there was in our experiments consistent differences between the titres in the cells and the blood serum on the one hand and lymph plasma on the other. The

cell and blood sera had similar titres, while the titres of the lymph plasma were always lower by from 1 to 4 tubes. This is in agreement with the results of other workers (see above).

There was no evidence of any change in the antibody titres of either the serum or the lymph cells during the 9 hours following the administration of colchicine. This extends the work of Fagraeus and Gormsen (1953). They did not find an increase in the antibody titres of blood serum in immunized mice receiving colchicine in spite of an accompanying lysis of lymphocytes and plasma cells.

Considering now the experiments on cell transfer, it may be seen from our results that the transference of unheated, washed, thoracic duct lymphocytes from immunized donor rabbits, (obtained during a secondary response) into normal unimmunized recipient rabbits, was followed by the appearance of antibody in the serum of the recipient. The recipient's serum titres rose from the 1st to about the 7th day, and began to decline on about the 11th day. By about the 21st day, the titres were not measureable. It should be stressed that none of these recipient rabbits had naturally occurring agglutinins to S. typhi (H-antigen) even in 1:2 dilution.

The obvious question which arises when considering these results is: what is the source of the antibody in the sera of the unimmunized recipient rabbits?

First, preformed antibody in the cells of the thoracic duct lymphocytes from the immunized donor may be transferred into the recipient rabbit and thus produce in it a passive immune response. If this were true, one would expect to find antibody in the sera of the control rabbits receiving the thoracic duct lymphocytes killed by heating to 56°C for half an hour - a procedure which kills the cells but in no way damages any antibody (Boyd, 1956) present in the cell at the time of transfer. In fact there was, in 6 out of 7 rabbits, some indication of a passive immune response, as a very low antibody titre could be demonstrated in the sera of the control rabbits on the 1st day after the injection of the thoracic duct cells. But this titre is not maintained for any length of time, nor does it increase in the control animals; in all these experiments the titres of blood serum in the control animals was after the 1st day, from 2 to 8 times lower than the animals receiving unheated cells. The non-persistence of the antibody titre and the failure for the antibody to increase in these control animals would seem inconsistent with the idea that the antibody in the recipient rabbits receiving unheated lymphoctyes from an immunized donor can be due to a transfer of preformed antibody contained in the suspension of the lymphocytes injected.

The second possibility for the source of the antibody

in the serum of the recipient, could be a transfer of antigen, either in its original or in a modified form, i.e. a process of active immunization. This possibility can be however excluded by the following arguments. The time-curve of the appearance of antibody in the recipient's serum after the transfer of the cells does not correspond to what one would expect in active immunization. The serum titres rise from the 2nd day onwards, while in active immunization the titres rise, at the earliest, from the 3rd or 4th day. Moreover if the appearance of the antibody in the serum of the recipient is to be interpretated as due to the process of active immunization, the same level of antibody titres should be seen in the control animals receiving the heat-killed cells, as the antigen is left undamaged by the process used for killing the cells. In the pair of recipient rabbits, A4 and K4, there was some suggestion that antigen had been transferred; in the control, the antibody titres rose to 64 on the 6th day. Even in this experiment however the animal receiving unheated cells had a titre 6 tubes higher than the control. (It is interesting to note that the pair of recipients A5 and K5 received cells from the same donor as A4 and K4, yet a considerable difference of the blood serum titres in the two pairs was obtained. The cells used in A5 and K5 were obtained from the thoracic duct later than the cells used in A4 and K4.)

The third explanation regarding the source of the antibody

in the recipient is that the transplanted, unheated lymphocytes are living cells and carry with them a mechanism of antibody-formation to the tissues of the recipient. The transplanted cells may multiply in the host's tissue and the progeny (perhaps plasma cells) may continue to produce antibodies for some time. If this is the case, then the significant cell, in the thoracic duct lymph would be the large lymphocytes, for only these appear from our and other experiments to be capable of cell division. This homotransplantation however cannot survive for longer than a few days because the host tissues will develop an immunity to the donor cells which will then be destroyed (Billingham, Brent and Medawar, 1954). The agglutinins to S. typhi (H-antigen) then progressively disappear from the host. The serum titres in the recipient begin to decline between 9 and the 11 days after transfer of the lymphocytes at a time when, presumably, the homograft reaction sets in: this is consistent with the explanation given above.

It may be, however, that the transferred lymphocytes themselves, without further multiplication, survive to produce antibody. This would be in agreement with the work of Wesslen (1952) who has shown that thoracic duct lymphocytes will, if derived from an animal infected with S. typhi, continue to produce antibody in vitro.

As a special case of adoptive immunity, it is theoretically possible, but less likely on general grounds, that the transferred living lymphocytes may in some way induce the antibody-producing cells of the host to form the antibodies. This could however be decided, if the recipient were irradiated by a suitable dose of X-rays - a process which depresses the antibody-forming mechanism; if adoptive immunity could be induced by transferring lymphocytes to such an animal, then the antibody will probably have come from the transferred cells or their descendants.

The present experiments are most convincingly explained if it is assumed that some of the transferred lymphocytes, possibly those capable of multiplication stay alive, and continue to produce antibody until the host rejects them by a homograft reaction.

SUMMARY AND CONCLUSIONS

1. The anatomy of the termination of the thoracic duct in rabbits is described.

2. At operation, the thoracic duct was exposed and divided; and uncontaminated lymph obtained.

3. The anti-mitotic alkaloid, colchicine, given intravenously in doses of 2.5mg./kg. body weight, was found to increase the output of both large and small lymphocytes 2 to 3 hours after the injection, usually without affecting the rate of flow. That there was no significant decrease in the output of thoracic duct lymphocytes for 10 hours following colchicine suggests that the majority of thoracic duct lymphocytes are not formed in the preceding few hours. This suggestion is in agreement with the observation that only up to 2.5% of the cells of the thoracic duct were in arrested mitosis following colchicine. Mitoses were never observed in small lymphocytes. It is thought that large lymphocytes are capable of division while small lymphocytes are not. Nuclear abnormalities, karyolysis and pyknosis, were seen in all types of lymphocytes and these abnormal forms were also present in the blood. Cells having mitotic figures were however not seen in the blood; some evidence is presented that these were trapped in the pulmonary circulation.

4. A profound granulocytopaenia occurred in the first hour following colchicine injection. In unanaesthetised

animals this was followed by a granulocytosis. In anaesthetised or anaesthetised and operated rabbits, the granulocytopaenia persisted, though anaesthesia or operation by themselves were found to produce a granulocytosis. The cause of this altered reaction remains obscure.

5. Colchicine, anaesthesia and operation all produced a fall in the level in the blood lymphocyte.

6. Occasional normoblasts were found in the peripheral blood after colchicine.

7. The spleens of rabbits receiving colchicine were smaller than controls.

8. The number and morphology of small and large lymphocytes of the thoracic duct in rabbits immunised against S. typhi did not appear to differ from the normal. The morphology of the thoracic duct lymphocytes after colchicine was the same as in unimmunised rabbits.

9. Preliminary observations suggest that the number of large lymphocytes in the peripheral blood of immunised rabbits is increased.

10. Antibody titres of thoracic duct lymph plasma were consistently lower than those of blood serum and of thoracic duct lymphocytes, estimated after homogenization. Colchicine administration did not alter these titres.

11. Thoracic duct lymphocytes were taken from immunised rabbits and injected, unheated, into normal rabbits.

Antibody appeared in the recipient, and the titre rose during the next few days. The titre began to fall at about the 9th day. Control transfers of cells taken similarily but heated to 56°C for 30 minutes produced in most rabbits only a transient titre in the recipient, the antibody level in these rabbits was, after the first day, always significantly lower than that in rabbits receiving unheated cells. It is thought that the transferred, unheated cells, or their progeny, continued to produce antibody until the homograft reaction destroyed them.

ACKNOWLEDGEMENTS

It was on the suggestion of Dr. K.B. Roberts, that these studies were undertaken and I am much indebted to him for his advice and help. My thanks are also due to Professor D. Whitteridge, F.R.S. for providing me with every facility and encouragement to carry on my work in the Physiology Department.

I wish to thank the technical staff of the Department, Miss M. Sturrock, Miss D. O'Hara and Mr. T. May, for the able assistance rendered.

I was favoured with the capable services of Mr. G. Renwick for the photography of the graphs and Mr. T.C. Dodds, Medical Photography Unit, for the photomicrographs.

The manuscript was carefully typed by Miss G. Alsop, for which I am much obliged.

This work was carried out during the tenure of a Scholarship from the Government of Pakistan.

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